Biology of the Fireblight Beetle, *Acacicola orphana* (Erichson) (Coleoptera: Chrysomelidae), A defoliator of *Acacia dealbata* (Link.)

by

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Declaration

This thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of the thesis.

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Abstract

Acacicola orphana (Erichson) is a winter developing insect that severely defoliates its host trees, *Acacia dealbata* (Link.) and *A. mearnsii* (De Wild.). Both adults and larvae feėd on green bark and foliage, resulting in damage which may lead to tree death.

This study investigates the biology and ecology of *A. orphana*, focussing on its developmental biology, distribution and host-plant relationships.

At the commencement of this project a basic guide to the different stages of *A. orphana* was established. Developmental biology was examined in the laboratory, where development from eggs to adults was found to require 1266 DD > 4.4 °C. Field development studies suggested a lower threshold. Consequently, inaccuracies were observed when laboratory information was used to predict the timing of stages in the field. Management strategies therefore need to be based on field estimates.

Natural enemies of *A. orphana* identified were; a tachinid, *Lixophaga* sp., a braconid hyperparasitoid, *Meteorus* sp. and a fungal pathogen, *Beauveria bassiana*. Fourth instar mortality attributable to the tachinid was up to17%. *Beauvaria bassiana* was recovered from adults only and caused up to 12% mortality.

Geographical distribution of *A. orphana* was mapped throughout southeastern Australia. This information was then used to predict the distribution throughout Australia and globally using CLIMEX, a climate modelling package. Global mapping predicted populations could survive in African and Asian countries where some *Acacia* species (in particular *A. mearnsii*) are economically important.

On the Australian mainland, *A. orphana* was observed predominantly on *A. mearnsii*, rather than on *A. dealbata,* which was its main host in Tasmania. Thus, experiments examining oviposition, larval development and survival between *A. dealbata* and *A. mearnsii* were undertaken. Whilst both species experienced similar levels of defoliation in the field, larval development was 25% faster on *A. mearnsii*.

Acacia orphana and its interactions with *Acacia dealbata* were the main focus of this study. Consumption studies showed final instar *A. orphana* consumed 0.93 $g^{-1}g^{-1}day^{-1}$ and the efficiency of conversion was 40%. Initial hypotheses relating to bark feeding behaviour were disproved, with findings that green bark feeding does not occur due to a lack of foliage, nor does it enhance the foliage quality for the next generation of larvae.

Further investigations of host-plant interactions involved assessing first instar larval survival and defoliation on four different *A. dealbata* provenances. General differences in nutrition, colour and fluctuating asymmetry of the trees were also assessed. One provenance experienced significantly less defoliation. Phenotypic differences showed that low foliar nitrogen, low moisture and redder-coloured foliage were related to increased defoliation and larval survival. It was hypothesised that climate and environmental conditions primarily regulate the host-plant interactions of *A. orphana*.

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1. Introduction

1.1 Project background

Environmental concerns and changing industry needs have prompted the Australian Forestry sector to diversify its native hardwood plantations. Traditionally *Eucalyptus* species have been utilized for pulp and paper production, but *Acacia dealbata* (Silver wattle) and *A. mearnsii* (Black wattle) are two hardwood species which have been identified as alternative sources of high quality fibre for pulp and paper production (Batchelor *et al.*, 1970; Muneri, 1997). These species also have a higher basic density which increases production efficiency during batch digestion pulping compared to *Eucalyptus* (Logan, 1986; Muneri, 1997). Furthermore, *A. dealbata* and *A. mearnsii* are fast growing and have potential to be incorporated into agroforestry situations where their fast growth rate makes them suitable for site revegetation (Cremer, 1990).

Prior to recognition as a source of pulp for papermaking, *A. mearnsii* bark grown in southeastern Australia was exported to England during the early 1800s. Upon arrival, the tannins were extracted and used for leather tanning (Mitchell, 1850). Since then, *Acacia* species have been established in areas of Asia and Africa where tannin production is economically important. Some of the countries involved are Sri Lanka (Midgley and Vinekanandan, 1986), China (Zhigang and Minquan, 1986), Tanzania (Kessy, 1986), Zimbabwe (Gwaze, 1986; Muneri, 1997) and South Africa (Chaunbi, 1997). Plantations have also been established in Brazil (Stein and Tonietto, 1997). Other major uses of *Acacia* include timber products for construction and fuelwood for domestic fires (Boland, 1986; Kaumi, 1986; Stein and Tonietto, 1997).

Commonly known as the Fireblight beetle due to the burnt appearance of the host after defoliation, *A. orphana* is a small chrysomelid beetle recognised throughout Southeastern Australia for its ability to severely defoliate *A. dealbata* and *A. mearnsii*. Repeated severe defoliation of hosts may result in considerable growth loss and even tree death (Elliott and de Little, 1985). The burnt appearance is due to the chewing of both leaves and green bark from the trees. This feeding behaviour was recognised as early as 1911 (French, 1911; Froggatt, 1923), yet has not been researched in any detail. It has also been suggested that *A. orphana* may have been partly responsible for the demise of the tanning industry in Southeastern Australia (Searle, 1991).

Acacicola orphana belongs to the sub-tribe Paropsini, which consists of predominantly leaf feeding beetles. Some paropsines such as *Chrysophtharta bimaculata*, *Paropsis atomaria*, *Paropsisterna tigrina* and *Paropsis charybdis* have caused substantial damage to their hosts (Carne, 1966a; Styles 1970; Steven, 1973; de Little, 1979a; Maddox, 1995) and thus considerable research has been undertaken for these economic species. Minimal literature exists on other paropsines. Only one paper on the biology of *A. orphana* had been published prior to the commencement of this project (Elliott, 1978) and thus before investing in *Acacia* plantations in Tasmania, the forest industry was interested in learning more about this pest. Thus, this project had several important industry objectives aside from host-plant interactions, including geographical distribution and general ecology of *A. orphana*.

The main aims of the project cover three broad research areas. Firstly, the basic biology (including development in relation to temperature) and natural enemies were studied, since future management programs could rely on this information. In particular, information on the timing of insect development and natural enemies can be used to optimise any management measures. The second aim was to investigate the geographical distribution of the Fireblight beetle. A patchy or very local distribution of the beetle throughout Tasmania would enable site selection in less populated areas, thus reducing the risk of infestation and the consequent economic damage. Furthermore, a model derived from the distribution of *A. orphana* in Southeastern Australia was used as a predictive tool for the beetle in other locations outside the current known Australian distribution. The final aim was to study the host-plant interactions of *A. orphana* and determine the factors that contribute to defoliation. These are essential to pest management and form a large field of active theoretical research. Therefore, a substantial part of this thesis is devoted to host-plant interactions.

1.2 Summary of thesis chapters

The main sections of the thesis and their significance are outlined below.

Chapter 2 – Literature review

This chapter provides an overview of *A. orphana* and the sub-tribe Paropsina, to which it belongs. It addresses the taxonomic status of *A. orphana* and reviews paropsine general biology, lifecycles, natural enemies, distribution and host-interactions. Ecological theories relating to insect-host interactions are also considered. Much of this chapter has been published as Simmul and de Little (1999) (Appendix 1).

Chapter 3 – Basic biology

Minimal information is available regarding the different life stages of *A. orphana* and thus figures and descriptions in this chapter aim to provide a working guide to aid future studies of the Fireblight beetle. However, it is not a taxonomic revision because a revision was considered beyond the scope of the thesis and a formal review of the sub-tribe is currently underway elsewhere (C.A.M. Reid, Australian Museum, pers. comm.).

Chapter 4 – Developmental studies in the field and laboratory

The developmental biology of *A. orphana* under constant temperature conditions and at three locations in the field in Tasmania is reported in this chapter. Results from the field and laboratory are compared and possible causes for differences discussed. Mating behaviour and reproduction are also examined.

<u>Chapter 5</u> <u>– Natural enemies</u>

This chapter reports on parasitoids and fungal pathogens reared from *A. orphana.* A preliminary investigation into the percentage parasitism of late instar larvae by a tachinid parasitoid was conducted. The tachinid parasitism study has been published as Simmul and Clarke (1999) (Appendix 2).

<u>Chapter 6</u> <u>– Geographical distribution</u>

The geographical distribution of an insect is influenced by climate, natural enemies and location of the host species. The distribution of *A. orphana* and its host was assessed in surveys of Southeastern Australia, with emphasis on Tasmania. The survey information, combined with temperature parameters determined in Chapter 4 was used to generate a model predicting the potential geographical distribution of *A. orphana* throughout Australia and globally. This information is useful to predict regions where *A. orphana* could become a pest if it were accidentally introduced.

<u>Chapter 7</u> <u>- Host choice – *A. dealbata* or *A. mearnsii*?</u>

During the surveys in Chapter 6 it was observed that *A. orphana* fed on both *A. dealbata* and *A. mearnsii.* In this chapter, oviposition, survival and development of the Fireblight beetle on the different hosts, *A. dealbata and A. mearnsii,* is examined.

<u>Chapter 8</u> <u>– Herbivory of *A. dealbata*</u>

This chapter focuses on *A. dealbata* as this is the dominant host species for *A. orphana* in Tasmania. Feeding damage by the insect involves both chewing of foliage and green stem material and, has potential to severely reduce tree growth. Total consumption by larvae and larval choice for the different plant parts were assessed. The effect of feeding damage on the host's nitrogen levels was also examined and hypotheses explaining the severity of damage are discussed.

Chapter 9 – Intraspecific herbivory on trees within a single species – *A. dealbata*

To assess if different provenances or families of *A. dealbata* experience different levels of herbivory an intraspecific field trial was conducted. This field trial aimed to identify any less-susceptible groups of trees that may be used for commercial development of plantations. Multiple phenotypic traits of the trees were measured. Tree stress, as determined using fluctuating asymmetry, was also used to assess if environmental stress was affecting herbivory. Results suggest that several phenotypic characters are of importance in the herbivore-host interaction. It is recommended that breeding programs should identify individual trees for heritability studies rather than families or provenances.

Chapter 10 – General discussion

This chapter summarizes the significant findings of this thesis and highlights more areas that require investigation. It also relates the results of the different chapters into the wider body of paropsine knowledge and insect ecology in general, showing that in particular, abiotic factors appear to be of importance in the population ecology of *A. orphana*. Suggestions for management of *A. orphana* in *A. dealbata* plantations are presented.

2. Literature Review

2.1 Outline of the Paropsina with emphasis on *Acacicola orphana*

Acacicola orphana is one of several species within the family Chrysomelidae belonging to the sub-tribe Paropsina as detailed by Daccordi (1994), and more recently, Kelly and Reid (1999) (Figure 2.1). The insects in this group are commonly referred to as paropsines. The classification of the different genera within the paropsines is somewhat confused due to the complexity of the taxonomy as well as poor initial descriptions. Since the late 1800s the group has been re-organised several times, with the most recent re-classification published being that of Kelly and Reid (1999). They present the name *Acacicola* for the insect that this thesis is based on and note that it is synonymous with *Pyrgoides*. Further information (C.A.M. Reid, pers. comm.) suggests that *A. orphana* will be re-classified into the genus *Peltoschema* (Reitter) as this is the oldest valid available name for *Acacicola* (Lea).

Erichson (1842) provided the original description of *A. orphana*, which has been translated as follows;

'Oblong convex, pale shell covering, bright, the elytra channel-punctured, light golden yellow, the spaces in between very finely punctured with slight pricks, first before the middle, 4 and 6. On the base and apex with dark little lines – long, 2 lines.

Black antennae with the first four joins golden/reddish yellow. Head finely punctured dispersedly. Thorax short with rounded sides, perfect/unimpaired. The thorax is sparingly and very finely punctured, the sides with large pricks. Coleoptera (elytra?), moderately convex, channel punctured with the spaces in between sparingly punctured most finely of all. The side edge punctured closely and vigorously, light golden/reddish yellow, in the space between 1. Before the middle, 2. On the foremost and rearmost, 6. On the foremost, 7. On the rearmost dusky smearing, the first longer near the seam with the foremost very short.'

(Translation: C. Ryan, Classics Dept. UWA; latin version is located in Appendix 3.)

Table 2.1 - Outline of the Chrysomelidae with specific reference to the Paropsina and *Acacicola*.

Superfamily:	**Chrysomelidoidea**	(4200 species in Australia)
Family:	**Chrysomelidae**	(3000 species in Australia)
Subfamily:	**Chrysomelinae**	(>600 species in Australia)
Tribe:		
	1) Timarchini	(Northern hemisphere only)
	2) Chrysomelini	(4 subtribes)
Subtribe:		
	i) Entomoscelini	
	ii) Chrysolinina	
	iii) Chrysomelina	
	iv) Paropsina	(15 valid genera*, 2+ currently invalid)

Genus:

a) Xanthogramma	j) Paropsisterna
b) Rhaebosterna	k) Sterromela
c) Dicranosterna	l) Philhydronopa
d) **Acacicola/Pyrgoides**	m) Paropsides
e) Faex	n) Chondromela
f) Niliosoma	o) Chrysophtharta
g) Paropsis	p) Trachymela
h) Procrisina	q) Novacastria
i) Trochalodes	

*according to Kelly and Reid (1999). Daccordi (1994) includes other genera.

Erichson (1842) called the insect *Paropsis orphana*. During a subsequent examination of the genus *Paropsis* (now sub-tribe Paropsina), the different species were divided into four groups, based on the puncturation of the elytra and *Paropsis orphana* was changed to *Paropsis orphanula* (Chapuis, 1877). Between 1894 and 1901 Blackburn again revised the genus, examining the puncturation of the elytra in more detail. Upon re-examination, Weise (1901) constructed two sub-tribes, Dicranosternini and Paropsini. His key was based on thoracic characters including bristle pores in the corners of the thorax. He erected the new genus *Pyrgo* for the small paropsines including *Paropsis orphanula. Pyrgo orphana* was differentiated from other paropsines by the following;

Thorax with bristle pore in corners (size < 7mm)
Thorax with setal pore only in hind corners, claws from simple to toothed.

Aslam (1968) renamed *Pyrgo* to *Pyrgoides* after recognising that the genus *Pyrgo* was already pre-occupied by a mollusc. *Pyrgoides orphana* was recognised until Daccordi's re-classification to *Acacicola*. This occurred because Lea (1903) described a single species as *Acacicola* which upon re-examination by C.A.M. Reid (pers. comm.) and Daccordi was actually shown to be a normal species of *Pyrgoides*. As Lea's name is older than Aslam's it should take precedence. Hence, the insect is now known as *Acacicola orphana*. Furthermore, uncertainty is still present within *Pyrgoides/Acacicola* and it may be re-classified again (C. A. M. Reid, pers. comm.). For the purposes of this study, the name *Acacicola orphana* is used.

Within the genus *Acacicola* the actual number of species is not precisely known at this stage. This is due partly to the taxonomic confusion surrounding the genus. Van den berg (1980, 1982) lists 12 *Pyrgo* (= *Acacicola*) species, six of which are unnamed. Reid (1992) describes the pupa for a further three species. Thus 15 species are currently listed, but 6 are unnamed and may not be new species.

2.2 General biology and lifecycle of Paropsines

Detailed lifecycle information is known only for some species in the genera that include economic pests; *Paropsis*, *Chrysophtharta* and *Paropsisterna.* Some information on the lifecycle of *Trachymela* and *Acacicola* has been published, but little is known regarding the other genera.

Under field conditions mature *Paropsis* and *Chrysophtharta* adults emerge from overwintering or reproductive diapause in the spring (Greaves, 1966; Styles, 1970; Kile, 1974; Tanton and Khan, 1978) and feed on "flush" foliage. In sunny weather they fly short distances and feed, but on cool or windy days they drop from the foliage and shelter in leaf litter on the forest floor (Greaves, 1966).

In the majority of species, eggs are laid late in the spring or early in summer on new or one year old foliage (Greaves, 1966; Styles, 1970; Tribe and Cillie, 1997). If new growth is absent, females may refrain from ovipositing for 2-3 weeks until favourable growth has developed (Carne, 1966a). Eggs are laid in a variety of patterns, depending on the species (de Little, 1979a). For example, *P. atomaria* Olivier lays on the stems of terminal shoots or occasionally around the extremities of sharply pointed leaves. Eggs are laid in successive rings, aligned in rows parallel to the shoot axis, or following a slightly spiral path depending on whether or not the shoot circumference is an exact multiple of basal egg width (Carne, 1966a). The number of eggs laid by this species is negatively correlated with increasing twig diameter, suggesting that branch tips are preferred oviposition sites (Tanton and Khan, 1978). In comparison, *P. charybdis* (Stål) lays eggs on the underside of leaves (Styles, 1970), as does *A. orphana* (Elliott, 1978). Oviposition behaviour of insects in the genus *Trachymela* is different to that of other paropsines, as these insects oviposit into crevices on the trunk of the host *Eucalyptus* (de Little, 1979a, Tribe and Cillie, 1997). *Trochalodes* produces eggs on the tips of long stalks which hold the eggs high above the leaves and away from predators (Selman, 1994a). *Chrysophtharta lignea* (Erichson) is virtually ovoviviparous, with the larvae bursting through a thin membrane within minutes of being deposited on a leaf (de Little, 1979a).

Many female paropsines lay 500-1000 eggs in a 3-4 month season (Carne, 1966a; Greaves, 1966; Styles, 1970; de Little, 1983). Elliott (1978) found females of *A. orphana* laid an average of only 114 eggs each, but he did not note what size the egg batches were. Egg batch size averages range from 9.8 for *T. tincticollis* Blackburn (Tribe and Cillie, 1997) to 75.2 eggs for *P. atomaria* (Carne, 1966a) and suggest a wide variation between genera. Blueweiss *et al.* (1978) observed that fecundity was proportional to size in many species. This is also apparent in *P. atomaria*, as larger females have been observed to lay more eggs per batch than smaller females (Carne, 1966a). Females need to be inseminated periodically to lay fertile eggs, but may lay infertile eggs if this does not occur (Greaves, 1966). Some species of chrysomelid are apparently able to store sperm for long periods and use this for fertilisation of eggs after emergence from diapause (Tauber *et al.*, 1988a; Stevens and McCauley, 1989), although no research has been conducted regarding sperm storage in the paropsines. Sex ratios in the field of 1:1 have been recorded for *C. bimaculata* Olivier (de Little, 1983) and *T. tincticollis* (Tribe and Cillie, 1997). Elliott (1978) did not examine sex ratios in his general biology paper on *A. orphana*.

Eggs of *P. atomaria*, *C. bimaculata* and *A. orphana* hatch within three weeks in the field (Carne, 1966a; Greaves, 1966; Elliott, 1978). From within the egg, the larva concentrates pressure on the thorax so the hatching spines on each side of the body pierce the chorion. This creates two slits, through one of which it forces its way out (Cumpston, 1939; Greaves, 1966). After approximately 30 minutes, the larva becomes pigmented (Cumpston, 1939). Newly emerged larvae feed on egg-shells (Cumpston, 1939; Carne, 1966a; Greaves, 1966; Tribe and Cillie, 1997) and then disperse as a group to the new "flush" foliage of the tree (Cumpston, 1939; Greaves, 1966; Carne, 1966a; Styles 1970; de Little, 1983). Larvae of *T. tincticollis* hide in crevices or bark curls on the trees, either singly or gregariously depending on the size of the crevice, and emerge to feed on young foliage just before sunrise (Tribe and Cillie, 1997).

Larvae develop through four instars. For *P. atomaria* and *C. bimaculata* each stage takes 5-6 days (Carne, 1966a; Greaves, 1966). *P. charybdis* develops from egg to adult in 7-9 weeks (Styles, 1970). In contrast to this, *A. orphana* is a winter active insect and its larvae develop over a period of 7-8 months (Elliott, 1978).

Some larvae remain gregarious throughout the larval stages (for example, *P. atomaria*) (Carne, 1966a) whilst others such as *Paropsisterna tigrina* (Chapuis) (Maddox, 1995), *C. bimaculata* (Greaves, 1966) and *P. charybdis* (Styles, 1970) may disperse individually or in small groups following the first or second instars.

When larval feeding is completed, larvae of some species drop directly from the leaves or crawl down the tree stems to pupate in the soil below (Carne, 1966a; Styles, 1970; Elliott, 1978; Tribe and Cillie, 1997; Clarke *et al.*, 1998a). After burrowing into the soil to a depth of 2-8 cm, depending on the soil hardness (Elliott, 1978), larvae construct a pupal case consisting of earth particles (Cumpston, 1939). The pupal period lasts for up to three weeks (Greaves, 1966; Carne, 1966a; Elliott, 1978) with the newly formed adult emerging during summer.

Adults fly upon leaving the pupal cell, making short abrupt flights from one part of the tree to another (Cumpston, 1939). Then, in preparation for overwintering, they congregate on the host trees in large numbers, where intensive feeding and subsequent fat-body accumulation occurs (Cumpston, 1939; Greaves, 1966; Carne, 1966a; Styles, 1970).

During the overwintering or reproductive diapause phase, adult insects can be found sheltering behind bark, under leaves and stones on the ground or in splits and cracks in the wood of dead trees (Cumpston, 1939; Greaves, 1966; Carne, 1966a; Styles, 1970; Tanton and Khan, 1978).

Recent habitat studies have shown that more overwintering *C. bimaculata* adults can be found in clumps of cutting grass, *Gahnia grandis* than *Eucalyptus* leaf litter (Clarke *et al.*, 1998b), indicating a need for more research on overwintering sites of paropsines. Overwintering insects are not gregarious (Cumpston, 1939; Clarke *et al.*, 1998b) and on warm days individuals may emerge and make short flights, or remain motionless for long periods on leaves exposed to sunshine (Carne, 1966a).

Paropsisterna tigrina has a similar lifecycle to *Paropsis* and *Chrysophtharta* species, with Maddox (1995) reporting that in northern New South Wales, Australia, significant rainfall events appear to start each period of larval activity. This insect has two peak periods, a small spring population and a much larger more damaging autumn population. *A. orphana* differs from the other known genera, as adults of this species mate and lay eggs in the autumn, and the larvae develop through the winter months and pupate in spring. Adults emerge from pupation in early summer (Elliott, 1978). Little is known of the activity of adult *A. orphana* during the summer months, prior to mating and oviposition in autumn.

2.3 Geographical distribution of Paropsines

Paropsines are mainly confined to Australia, although some species are found in New Guinea (Selman, 1985), New Zealand (Styles, 1970) and South Africa (Tribe and Cillie, 1985, 1997). Accidental introductions have occurred into New Zealand, South Africa and recently several *T. sloanei* were discovered in southern California, USA where they have spread over an area of approximately 2500 square miles (T.D. Paine, UC Riverside, pers. comm.). Daccordi (1994) notes *Acacicola* and *Paropsides* in southern Asia and D. W. de Little (North Eucalypt Technologies, pers. comm.) has observed *Paropsides umbrosa* (Chapuis) feeding on flowers of Fabaceae species in Tasmania. *A. orphana* has been recorded feeding on *Acacia* species in Victoria (Searle, 1991) and Tasmania (Elliott, 1978). Van den Berg (1980) recorded *Pyrgo* *orphana* (= *Acacicola orphana*) as an occasional feeder on *A. saligna* in Esperance, Western Australia but this has not been confirmed.

Within Australia, most paropsine species are located in the southeast, which may be partly due to the enormous diversity of potential host species and climatic variation in this region. No attempts to model the geographical distribution of any paropsine insect based on climatic parameters has been made. The recent finding of *T. sloanei* in California suggests that these insects are able to establish rapidly in new environments after accidental introduction. An indication of the potential global distribution of *A. orphana* and other paropsines is useful to predict where the insects may become a problem if introduced. Potential distribution of insects may be modelled in relation to climate using a program called CLIMEX (Skarratt *et al.*, 1995). This program has been used to examine the potential distribution of a number of species including the European wasp, *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae) in Southern Africa (Tribe and Richardson, 1994); terrestrial planarians, which were deleterious to earthworm populations in Europe (Boag *et al.*, 1995) and the Queensland fruit fly, *Bactrocera (Dacus) tryoni* (Yanow and Sutherst, 1998). These studies all used the potential distribution generated from the program to suggest localities where the different pest organisms have potential to establish, thus enabling forward planning of management options.

2.4 Host Interactions

2.4.1 Host range

Paropsine beetles feed on a diverse range of plants including species of *Acacia*, *Leptospermum, Melaleuca*, *Angophora*, *Tristania*, *Callistemon*, *Eucalyptus* and *Corymbia*. These are all myrtaceous species, except for *Acacia*, which is leguminous. Little information exists on the host range and choice of the non-eucalypt feeding species, however *Acacicola* species have been observed feeding on a variety of both bipinnate and phyllodinous *Acacia* (Froggatt, 1923; Elliott, 1978; Van den berg, 1982; Hawkeswood, 1983; Searle, 1991; Reid, 1992).

Of the *Eucalyptus*-feeding paropsine species, many have a wide host range and whilst the non-eucalypt feeding paropsines may exhibit similar broad host ranges these have not been quantified. A single eucalypt-feeding species can feed and develop on a range of hosts but may be more frequently observed on some hosts more than others. This may partly reflect the number of available hosts within a region (Fox and Morrow, 1981). Alternatively, some insect species may show a high degree of specificity (Waldbauer, 1968; Bernays and Graham, 1988; Siemens *et al.*, 1991) which has the potential to change plant species composition over time as more favoured hosts are repeatedly defoliated (Burdon and Chilvers, 1974; Morrow, 1977).

Climatic parameters limiting the geographical distribution of the host may also affect the host range of an insect. If climate does not have a limiting effect on the insect's development then adaptation to an alternative host can lead to extension of its geographical range or allow it to exist in an area where its main host is uncommon. For example, the colorado potato beetle (*Leptinotarsa decemlineata* Say) has adapted to *Solanum elaeagnifolium* in regions where its natural host is not abundant (Hsiao, 1978).

2.4.1.1 Acacia dealbata *and* A. mearnsii *– Commonly observed hosts of* A. orphana

Acacia species were first described in the mid-1700s (Millar, 1754 - cited by New, 1984) and occur in most continents (Boland *et al.*, 1992). There are approximately 1000 recognised species and almost 700 of these occur in Australia. Numerous revisions of the genus have been conducted since 1754 (for details see New, 1984), however some confusion is still prevalent. There are currently three subgenera in the genus *Acacia* and *A. dealbata* and *A. mearnsii* are in the subgenus *Acacia* (= Series *Gummiferae* Benth.) The species in this subgenus are characterised by spinescent stipules, funicle with initial orientation anti-rapheal and pollen with pores. In particular, *A. dealbata* and *A. mearnsii* are morphologically characterised by compound bipinnate leaves consisting of a number of pinnae arranged along a central rachis. Extrafloral nectaries are present on the rachis. Detailed descriptions of both species are provided in Appendix 4.

Acacia dealbata is commonly referred to by the name Silver Wattle due to its pale silvery foliage. *A. mearnsii* is a darker green colour and is commonly referred to as Black Wattle (Figure 2.1).

Figure 2.1 – Foliage of *A. dealbata* (left) and *A. mearnsii* (right).

The phenology of *Acacia* species is not well studied. However, observations indicate flowering in Tasmania occurs late in winter to early summer. Trees have a period of rapid shoot growth during the spring. Both species are short-lived, having a life expectancy of approximately 20 years (Searle, 1997). During that time, *A. dealbata* can grow to a height of up to 30m and *A.*

mearnsii to approximately 15 m (Boland *et al.*, 1992).

Acacia dealbata and *A. mearnsii* are common species in the cool sub-humid to warm sub-humid regions of Southeastern Australia (Searle, 1997). *A. dealbata* occurs between latitudes of 29 to 43° S whilst *A. mearnsii* is located over a smaller regional area, between latitudes 34 and 43° S. *A. dealbata* is found at altitudes of 350-1000m ASL in Tasmania, but *A. mearnsii* has been observed only at altitudes of up to 850m ASL (Boland *et al.*, 1992). Both species are capable of growing at higher altitudes however, as plantations at 1500m ASL or higher are common in some African countries (Luyt *et al.*, 1986; Kessy, 1986; Kaumi, 1986). In Australia, the species are commonly observed growing in open-forests in association with *Eucalyptus* species or on cleared land.

Both species prefer fertile well-drained soils, but they will grow on well-drained, poorer soils. They are frost tolerant and temperatures above 40 °C are stressful and inhibit growth (Boland, 1986). Rainfall for optimum growth is approximately 600 – 1000 mm per annum (Boland *et al.*, 1992).

A range of insects have been observed feeding on *A. dealbata* and *A. mearnsii* (Van den berg, 1982; Hunt *et al.*, 1996), but no observations of defoliation as severe as that caused by *A. orphana* have been recorded.

2.4.2 Host location and selection

Location of a host requires either directed or random foraging by the insect. Feeding by insects on one tree may release chemical stimuli which attracts other insects to the area, thereby directing the foraging behaviour (Bach, 1980; Bolter *et al.*, 1997). Alternatively, insects which forage at random may alight on a number of plants that are then either accepted or rejected according to chemical or visual stimuli which affect the insect's ability to oviposit or feed

(Thorsteinson, 1960; Beck, 1965; Hsiao, 1969; Chapman, 1974; Tomlin and Borden, 1996).

Visual cues such as host apparency may direct foraging on a large or small scale. New (1988) outlined apparency as '*a number of different parameters related to how obvious the plant may be to the herbivores*'. For example, Feeny (1976) suggested that trees in a forest were less likely to be infested than individuals that were exposed. Similarly, where there is a high diversity of plant species a single host has a greater chance of being overlooked (Root, 1973 – "*resource concentration hypothesis*"; Bach, 1980). Plant architecture (Lawton, 1983; Strauss, 1988), vigour (Price, 1991 – "*plant vigour hypothesis*"), colour or spectral quality (Thorsteinson, 1960; Prokopy and Owens, 1983) and chemical stimuli (Chapman, 1974; Bach, 1980; Tomlin and Borden, 1996) may also affect host selection.

Following host location and selection, the process of oviposition requires a series of behavioural events regulated by chemical stimuli (Beck, 1965; Hsiao, 1969). It is generally hypothesised that ovipositing females are able to discriminate between hosts and choose those that will maximise larval survival (Jaenike, 1978; Kouki, 1993; Hanks *et al.*, 1993; Hanks *et al.*, 1995a; Carr *et al.*, 1998) but this is not the case for all species. For example, *Spodoptera exigua* (Hubner) (Lepidoptera:Noctuidae) preferred to oviposit on *Chenopodium murale*, yet the larvae showed superior performance when fed *Apium graveolens* (Berdegue *et al.*, 1998). De Little (1983) hypothesised that insects feed on hosts which provide the fastest development and reduce their potential for predation. It is also possible however, that selection for less preferred hosts may occur in some insects that are escaping predators (Berdegue *et al.*, 1998) suggesting that top-down factors may also affect host interactions to some degree. One example is *Neodiprion sertifer* (Geoffroy) larvae which consume bark of their host tree to increase their defensive abilities at the expense of development time (Larsson *et al.*, 1986). Physical characteristics of the foliage such as leaf toughness and hairs can also affect the ability of larvae to feed, but may not inhibit oviposition (Beck, 1965; Feeny, 1970; Larsson *et al.*, 1986) as the stimulants for oviposition and feeding are not necessarily the same. In particular, if feeding stimulants are absent larvae may not feed on a plant (Hsiao, 1969). This may partially explain why larvae do not always perform best on the host that the ovipositing female selects.

Within the paropsines, larval performance has been found to vary between eucalypt species as shown by Carne (1966a) who observed larvae of *P. atomaria* matured faster when feeding on *E. blakelyi* than on *E. maculosa*. S.C. Baker, J.A. Elek and S.G. Candy (unpublished data) found larvae of *C. bimaculata* developed faster and consumed less foliage of the *Symphyomyrtus* species *E. nitens*, than those on the *Monocalyptus* species *E. regnans*, even though adults oviposit on expanding leaves of *E. regnans* when given a choice between the two species (Steinbauer *et al.*, 1998). They found an efficiency of conversion (EC) of food ingested into body mass (see Chapter 8) of 24% for fourth instar larvae fed with *E. nitens* and only 13% for *E. regnans* foliage, suggesting that *E. nitens* is a better food source. The oviposition choice for the apparently poorer larval food source may be due to the faster growth rate of *E. nitens* which results in a smaller canopy with less expanding leaves for oviposition (Steinbauer *et al.*, 1998). De Little and Madden (1975) found *C. bimaculata* and *C. agricola* (Chapuis) consumed both *E. dalrympleana* and *E. delegatensis* foliage, but survival of *C. bimaculata* larvae was significantly less on *E. dalrympleana*. They observed that both species consumed more and matured faster on *E. delegatensis*, but when provided with a choice, *C. agricola* oviposited on *E. dalrympleana*, possibly indicating adaptation to an alternative host to avoid resource competition. Whilst *A. orphana* has been observed feeding on a variety of *Acacia* species (Froggatt, 1923; Elliott, 1978; Van den berg, 1982; Searle, 1991; Reid, 1992), its oviposition choice and larval development of the insects on different hosts has not been examined.

Leaf chemistry influences the feeding behaviour and development of insects (Feeny, 1970; Li, 1993). Foliar nitrogen content has been related to larval development of paropsines (Fox and Macauley, 1977; Morrow and Fox, 1980; Ohmart *et al.*, 1987). Patterson *et al.* (1996) found leaf toughness and nitrogen levels were relatively unimportant for survival and growth of *C. bimaculata* larvae on young foliage. However, when fed on older, tougher leaves which are lower in nitrogen, Ohmart *et al.* (1987) found that *P. atomaria* larval growth slowed below a threshold concentration of 1.7% N. They suggested that early instar larvae may have difficulty feeding on these leaves due to toughness and that while later instar larvae may be able to feed on these leaves, they may need to consume more foliage to meet their nutrient requirements. *A. dealbata* and *A. mearnsii* are N-fixing legumes and hence it would be unlikely that nitrogen would be limiting larval growth on these species. High foliar 1,8-cineole levels have been found to correlate with reduced paropsine herbivory in *E. camaldulensis* (Stone and Bacon, 1994) and *E. obliqua* (Li, 1993). However, no effects on larval feeding rates have been found for condensed tannins and other phenols (Fox and Macauley, 1977).

The water status of a plant may also affect insect feeding and survival. Moisture levels of 70% or higher have been found to be optimal for larval performance (Scriber and Slansky, 1981; Harrell *et al.*, 1982). Both moisture and nitrogen levels in foliage decrease as it ages and becomes tougher (Scriber and Slansky, 1981).

2.4.3 Effect of feeding on the host

Insect feeding may enhance the tree as a resource for insects above that of trees that have never been defoliated (Stenseth, 1978; Hartvigsen *et al.*, 1995). Insect feeding early in a season may also enhance the host as resource for insects later in the season, as the tree produces nutritious regrowth following defoliation (Craig *et al.*, 1986 – *"resource regulation hypothesis"*). This has been shown by Roininen *et al.* (1988) who found the bud-galling sawfly, *Euura mucronata* (Hartig) enhanced its host tree through stimulation of new growth. This meant that nutritious juvenile foliage was available for subsequent generations. Also, trees which are stressed as a result of moisture deficiency or prior insect attack have been found to have higher nitrogen levels in their foliage which is attractive to insects (White, 1969; Ohmart *et al.*, 1985a). It is difficult however, to fully assess the deleterious effect of the defoliation on many of the developmental aspects of the tree's lifecycle including seed production (Kulman, 1971) and photosynthetic capacity (Leon, 1989). Repeated severe defoliation over many years may also negatively affect the trees by reducing their ability to recover, as the plants compensate for the defoliation at the expense of the roots (Blossey and Schat, 1997). Severe herbivory may also indirectly promote species richness in an area by increasing light availability to suppressed understorey species (Carson and Root, 2000). Furthermore, on a tree level defoliation of only parts of many leaves or entire defoliation of only a small number of leaves will have different effects on the ability of the tree to recover (Floate *et al.*, 1993) and over a period of years may change the shape of a plant (Doak, 1992).

The effect of feeding is also related to the timing of the defoliation (Myers and Post, 1981; Doak, 1992). Defoliation during winter when plant tissues are less active may have less effect on the tree than defoliation when the tree is actively growing (Carne, 1969; Mazanec, 1974) or may further enhance the effects of unfavourable climatic conditions on the tree. Whilst the apical foliage of eucalypts may re-grow after defoliation by paropsine larvae, full recovery is often prevented by adult feeding prior to winter and thus the damaged upper portion of the crown is exposed to further injury from frost damage during winter (Elliott *et al.*, 1993).

2.5 Feeding behaviour of Paropsines

Adult and larval eucalypt feeding behaviour and patterns are distinctly different (Cumpston, 1939; Greaves, 1966; Kile, 1974). Adults feed alone, chewing from the edge of a leaf towards the midrib which results in a scalloped effect on the leaf margins (Greaves, 1966; de Little, 1983). Large numbers of beetles result in the host tree appearing ragged and defoliation in successive seasons can result in the tree developing a "broom-topped" appearance (Leon, 1989;

Elliott *et al.*, 1993). Intense adult feeding early in the season promotes development of "flush" foliage which is the preferred food source for larvae developing later in the season (Selman, 1994b). The most vigorous feeding by adults is just prior to overwintering (Cumpston, 1939; Carne, 1966a; Styles, 1970) which may prevent re-foliation of branches in the upper parts of the crown, leaving, in some areas, exposed shoots susceptible to further damage from severe cold in winter as previously mentioned (Elliott *et al.*, 1993).

Paropsine larvae feed preferentially on new growth, stripping the host trees of young leaves and shoots, both apically and laterally (Cumpston, 1939). Branches become progressively defoliated as the larvae grow and move on to older and less succulent leaves (Greaves, 1966; Carne, 1966a). As larvae develop, their rate of consumption rises steeply (Carne, 1966b). For example, Greaves (1966) found the two later instars of *C. bimaculata* caused 90% of defoliation even though they were present for only 55% of the total larval period. It has been hypothesized that as larvae age, they become less selective feeders, and therefore consume a higher amount of indigestible fibre in their diets (Mukerji and Guppy, 1970; Lawton, 1971; Kogan and Cope, 1974). This appears relevant to the paropsines, with some authors observing larvae of *C. bimaculata* and *A. orphana* consuming green bark of the host trees when all foliage has been consumed (Greaves, 1966; Elliott, 1978). Whilst *A. orphana* has been observed to feed on both leaves and stem material, this behaviour does not appear to extend to the other *Acacicola* species which are predominantly flower feeders (Reid, 1992; Hawkeswood, 1983; Hawkeswood, 1994). The importance of flowers in the diet was shown by New (1981) who found that day-old pupae of *Acacicola hamadryas* (Stål.) (formerly *Pyrgoides*) fed on *A. baileyana* flowers alone were heavier than those that had fed on foliage.

2.6 General Behaviour

2.6.1 Defence behaviour

Defence behaviour in paropsines has been widely observed (Cumpston, 1939; Greaves, 1966; Carne, 1966a; Moore, 1967; Tribe and Cillie, 1997). Adult paropsines feign death and drop to the ground if the foliage on which they are resting is disturbed (Greaves, 1966; Edwards, 1982). They are able to protect themselves from some smaller predators (for example, predatory ants) by pulling their legs under the body and clinging tightly to the leaf surface (Selman, 1994b).

The main paropsine larval defence mechanism is a pair of glands, present in each larval stage and everted from the base of the 8th tergite (T8) when the larva is threatened (Moore, 1967; Tribe and Cillie, 1997). These glands are particularly well developed in the larvae of *Paropsis* and *Chrysophtharta* (Selman, 1994b). Carne (1966a) elaborated on this defence mechanism, having observed three levels of response to predator disturbance by *P. atomaria* larvae. Slight disturbance causes larvae to stop feeding, raise their abdomens and tap on the leaf. This behaviour continues for several minutes, until the threat retreats. Violent disturbance (for example, a bird disturbing the foliage) causes larvae to regurgitate a drop of yellow fluid. If the predator contacts the larvae they vigorously flex their abdomens so that the everted abdominal glands on T8 come into contact with the attacker. These glands secrete a fluid containing hydrogen cyanide (Moore, 1967) which is toxic to predators (Cumpston, 1939; Greaves, 1966). Ants, for example *Iridomyrmex rufoniger* (Lowne) and *I. detectus* (Smith), were found to die within minutes of being struck (Carne, 1966a). If the predator withdraws, the glands retract. Up to 10 minutes may pass before the larvae return to normal feeding or resting behaviour (Carne, 1966a). Larvae of the genera *Trachymela* and *Sterromela* have less well developed defence glands and rely instead on cryptic colouring to camouflage them from predators (Selman, 1994b).

Defensive grouping behaviours have been given the terms 'cycloalexy' and 'alytoalexy'. Vasconcellos-Neto and Jolivet (1988) used the term cycloalexy to describe the formation of larvae in a circle for defence purposes. This circle may often contain larvae of different developmental stages and species. Selman (1994b) suggested the term alytoalexy for larvae which form into defensive rows, usually in the angle between a lateral shoot and a stem of the host plant.

When not under threat from predators, some paropsine larvae still exhibit grouping behaviour. Initially larvae are gregarious and this may last throughout the four stages as in *P. atomaria* and *P. tasmanica* (Baly) (Carne, 1966a; de Little, 1979b). Species not gregarious in the later stages may still reform into groups to rest and/or migrate. This grouping behaviour may be diagnostic of some species. For example, *Chrysophtharta obovata* (Chapuis) (= *C. variicollis* Chapuis) are gregarious, arranging themselves on the leaf in a circular fashion with their heads pointing inward toward a central focal point (Cumpston, 1939). *Paropsisterna liturata* (Marsham) (Cumpston, 1939) and *Paropsis rubidipes* (Blackburn) (de Little, 1979b) are almost solitary in habit, as are larvae of *Trachymela* and *Sterromela* which feed individually and are nocturnal. *Trachymela* and *Sterromela* larvae rest under bark during daylight (de Little, 1979a; Tribe and Cillie, 1997).

2.6.2 Flight behaviour

Adult flight behaviour can result in groups of insects moving large distances in a single season. *P. charybdis*, *P. atomaria* and *C. bimaculata* adults have been especially observed to fly during warm summer days (Greaves, 1966; Carne, 1966a; White, 1973; Clarke *et al.*, 1997). Adults of *C. bimaculata* dwell in an area for approximately 10 days, during which time they feed and lay eggs. Before the eggs hatch, the adults move to another area (Clarke *et al.*, 1997). This behaviour results in movement over several kilometres. In Tasmania, Australia, no genetic differences were observed between populations of *C. bimaculata* greater than 160 km apart, suggesting the insects had dispersed over this distance (Congdon *et al.*, 1997). In South Africa, *Trachymela tincticollis* (Blackburn) has been observed to move average distances of 130km annually, with dispersal over 200km in one area (Tribe and Cillie, 1997). Wind may also aid dispersal over long distances as suggested by White (1973) when discussing movement of *P. charybdis* from one island to the other in New Zealand.

2.6.3 Pre-pupation behaviour

Recently Clarke *et al.* (1998a) attempted to quantify an unusual behaviour first noted by Greaves (1966) who made the observation that larvae having completed their development *"fall to the ground"* from the canopy of the host trees. Clarke *et al.* (1998a) supported this statement, having found final instar larvae of *C. bimaculata* falling from trees 50-65m in height. It appears that to accumulate enough food reserves for pupation the larvae consume food until they are unable to grip the leaves. At this stage they simply drop from the trees to the ground where they burrow into the soil to pupate (A.R. Clarke, Griffith University, pers. comm.).

2.7 Natural Enemies

In ecological theory, predators and parasitoids have been recognised as top-down factors that can limit the amount of herbivory experienced by trees. In particular, natural enemies have an important influence on the mortality of paropsine beetles with authors reporting up to 97% mortality from egg to adult stage of *C. bimaculata* and *P. atomaria* (Greaves, 1966; Tanton and Khan, 1978; de Little, 1982; Tanton and Epila, 1984; de Little *et al.*, 1990; Mo and Farrow, 1993). Natural enemies can be divided into three categories; a) egg parasitoids, b) larval parasitoids and c) predators. Predators may consume eggs, larvae or adults.

Paropsine insects are also susceptible to fungal pathogens (de Little, 1979a), nematodes

(Selman, 1989) and bacteria. In particular, a bio-insecticide developed using *Bacillus thuringiensis* has shown potential as an alternative to chemical insecticides in Coleoptera (Harcourt *et al.*, 1996; James *et al.*, 1998) and more specifically *C. bimaculata* (Beveridge and Elek, 1999).

2.7.1 Egg parasitoids

All egg parasitoids recorded are hymenopterous species, and caused a maximum of 20% mortality in *P. atomaria* (Tanton and Khan, 1978). Parasitised eggs are dull brown in appearance, instead of glossy (Tanton and Khan, 1978). Insect parasitoids of the Paropsini are listed in Table 2.2. Other egg parasitoids observed include three unknown genera reared from *P. atomaria* (Tanton and Khan, 1978; Tanton and Epila, 1984).

Table 2.2 - Parasitoids of Paropsines.

Host	Stage	Parasitoid	Reference
P. atomaria	Egg	*Neopolycystus insectifurax* (Girault) (Pteromalidae)	1, 2, 7
	Egg	*Enoggera* sp. (Pteromalidae)	7, 11
	Egg	*Aphaneromella ovi* (Dodd) (Playtgasteridae)	7, 1, 2
	Egg	*Baeoanusia albifunicle* (Girault) (Encyrtidae)	7
	Larval	*Froggattimyia anguliventris* (Mall.) (Tachinidae)	1
	Larval	*Froggattimyia tillyardi* (Mall.) (Tachinidae)	1
	Larval	*Paropsivora* sp. (Tachinidae)	1
	Larval	*Eadya paropsidis* (Braconidae)	2
P. charybdis	Egg	*Neopolycystus insectifurax*	8
	Egg	*Enoggera* sp.	8, 9
C. obovata (=*C variicollis*)	Egg	*Neopolycystus insectifurax*	7
	Egg	*Enoggera* sp.	7
C. bimaculata	Egg	*Enoggera* sp.	4, 11
	Egg	*Aphaneromella* sp.	4
	Larval	*Anagonia rufifacies* (Macquart) (Tachinidae)	3, 4
	Larval	*Paropsivora* sp.	3, 4
	Larval	*Eadya paropsidis*	3
T. tincticollis	Egg	*Neopolycystus insectifurax*	10
	Egg	*Enoggera reticulata* (Naumann)	10, 11
	Egg	*Enoggera nassaui*	11
A. orphana	Larval	*Lixophaga* sp. (Tachinidae)	5
	Larval	*Deltomyza australiensis* (Malloch) (Tachinidae)	5
Trochalodes pedestris	Larval	*Anagonia scutellata* (Malloch) (Tachinidae)	6
Paropsisterna beata	Egg	*Enoggera nassaui*	11

Reference key: [1]Tanton and Khan, 1978; [2]Tanton and Epila, 1984; [3]de Little, 1982; [4]de Little *et al.*, 1990; [5]Elliott, 1978; [6]Cantrell, 1986; [7]Mo and Farrow, 1993; [8]Bain and Kay, 1989; [9]Kay, 1990; [10]Tribe and Cillie, 1997; [11]Naumann, 1991.

2.7.2 Larval parasitoids

Tachinids account for up to 62% of the mortality caused by parasitism in paropsine larvae (Greaves, 1966; Tanton and Khan, 1978; Tanton and Epila, 1984; de Little *et al.*, 1990). Tachinids lay a small, white, oval egg on the cuticle of the host larva (Greaves, 1966; Elliott, 1978; de Little, 1982) from which the parasitoid larva emerges and burrows into the body of the host (Greaves, 1966). Parasitoid eggs may not always be apparent on the cuticle, as they are lost with the cuticle when the insect moults (de Little, 1982). Usually only one parasitoid larva develops inside the host (Tanton and Epila, 1984; Cox, 1994), although Elliott (1978) noted up to five eggs on some host *A. orphana* larvae. The parasitoid does not kill the host until it nears the prepupal stage, at which time parasitised larvae become darker in colour. The parasitoid then forms a puparium either inside the host cuticle that may protrude from the dead host (Greaves, 1966; Tanton and Khan, 1978), or it may bore a hole to exit the host prior to pupation. It has also been hypothesised that parasitoids that pupate inside the body of the host may be more susceptible to attack from hyperparasitoids (Tanton and Khan, 1978).

Parasitoids attack all larval stages, but some appear to parasitize some stages more than others. An example is the braconid *Eadya paropsis* (Huddleston and Short) which achieved maximum levels of parasitism by the second larval instar in field collected *C. bimaculata* larvae (de Little, 1982). In this paper, de Little lists the parasitism percentages as ranging between 30.7 and 41.6% but does not note if this observation has statistical significance. De Little (1982) also suggests that the tachinid, *Anagonia rufifacies* (Macquart) preferred fourth instar hosts for oviposition, while *Paropsivora* sp. exhibited little differentiation between host stages.

Eadya paropsidis is the dominant larval parasitoid of *C. bimaculata* and *P. atomaria* (de Little, 1982), causing up to 93% parasitism (Tanton and Epila, 1984). Usually six eggs are laid per host, although as many as 13 eggs have been observed (Tanton and Epila, 1984) however, it is likely that only one egg would successfully develop into an adult. Tanton and Khan (1978) incorrectly identified this as *Aridelus* sp.

2.7.3 Hyperparasitoids

Hyperparasitoids have also been identified from tachinid parasitoids (Tanton and Khan, 1978;

de Little, 1982; Tanton and Epila, 1984). Tanton and Epila (1984) found tachinid larvae within *P. atomaria* containing 1-4 larvae of the ichneumonid *Mesochorus* sp. Also identified was *Perilampus tasmanicus* (Cameron) (Pteromalidae), a hyperparasitoid with high reproductive capacity and an ability to parasitize both braconid and tachinid hosts.

2.7.4 Nematodes

Another natural enemy was observed by Selman (1989) who found nematodes of the family Mermithidae infesting populations of paropsine beetles. These nematodes parasitise the larval stages of the host and emerge either from final instar larvae or adults. If they emerge from larvae, the larvae are killed, but in fast developing, multivoltine species, adult nematodes may emerge from adult beetles without causing death. Initially host beetles appear sick and discoloured, but under laboratory conditions they have been observed to recover and lay eggs.

2.7.5 Predators

Insect predators cause substantial loss of paropsine eggs and early instar larvae (Elliott and de Little, 1980; Selman, 1989). De Little *et al.* (1990) recorded up to 77.6% predation of eggs at one site in the north-west of Tasmania, Australia and observed that the main predator was a coccinellid, *Cleobora mellyi* (Mulsant). *C. mellyi* and *Harmonia conformis* (Boisduval) another coccinellid, have been noted to consume eggs of *P. atomaria* (Mo and Farrow, 1993; Baker, 1998), *P. charybdis* (Bain *et al.*, 1979), *C. variicollis* (Mo and Farrow, 1993) and *C. bimaculata* (Greaves, 1966; de Little *et al.*, 1990; Mensah and Madden, 1994). Greaves (1966) found that they also consumed early instar larvae and are predators in both the larval and adult stage. Other important predators include the cantharid *Chauliognathus lugubris* (Fabricius) which feeds on the eggs, larvae and pupae of *C. bimaculata* (Mensah and Madden, 1994 - wrongly identified as *C. pulchellus*; Shohet and Clarke, 1997) and the pentatomid *Oechalia schellenbergii* (Guérin-

Méneville) which consumes eggs of *P. atomaria* (Mo and Farrow, 1993) and *P. charybdis* (Bain and Kay, 1989). Minor predators observed feeding on *P. charybdis* are *Cermatulus nasalis* (Westwood) (Pentatomidae), *Vespula germanica* (Fabricius) and birds (Styles, 1970).

2.7.6 Pathogens

Bacillus thuringiensis var. *tenebrionis* has been successfully used to reduce numbers of early instar *C. bimaculata* larvae in trials in Tasmanian *Eucalyptus* forests (Elliott and Greener, 1993). This biological insecticide has little effect on non-target, natural predator species such as *Chauliognathus lugubris* (Beveridge and Elek, 1999) and thus has potential to be a successful bio-control spray. As its activity is limited to first and second instars however, improvement in the formulation is required before it becomes an effective management option.

Entomopathic fungi are often easily propagated and stored, and are thus attractive as bio-insecticides (James *et al.*, 1998). De Little (1979a) identified *Metarhizium* sp. from *C. bimaculata*. *Beauveria bassiana* has also been found to reduce numbers of *C. bimaculata* (Elliott and Greener, 1993). This pathogen requires both high humidity as well as temperatures of 25-32 °C (James *et al.*, 1998) which are uncommon in Tasmania for much of the year. It is able however, to survive in the soil for up to 120 days (Anderson *et al.*, 1988) and thus has potential to infect Tasmanian paropsines during their pupal stage, but may not become apparent until later in the insects lifecycle when weather conditions are more favourable. *B. bassiana* has been used successfully on the Colorado potato beetle and has shown potential as a bio-insecticide for this species (Anderson, *et al.*, 1988).

2.8 Pest Potential

Paropsine adults and larvae are capable of causing considerable damage to their host trees (Carne, 1966a; Greaves, 1966; Styles, 1970; Elliott, 1978; de Little, 1983; Tribe and Cillie, 1997). The main damage to commercial plantations caused by paropsine pests is the loss of pulpwood and timber production resulting from heavy and repeated defoliation of the host trees (Carne 1996a; Bain and Kay, 1989; de Little, 1989; Neumann, 1993). Many of the observations of herbivory are from plantation *Eucalyptus* species where damage caused by paropsine pests results in economic losses. For example, severe defoliation in a *Eucalyptus nitens* plantation by *C. bimaculata* was estimated to result in the loss of two years growth or 22 m^3/ha (de Little, 1989). Elliott *et al.* (1993) found that defoliation of one year old *E. regnans* trees resulted in a 55% reduction in height compared to undamaged (protected) trees. After eight years, Elek (1997) observed that the same insect-protected trees were 25% taller than unprotected trees, with almost three times the wood volume. Details of damage to non-eucalypt species are uncommon in the literature, but Elliott (1978) reported that 34 out of 200 *Acacia dealbata* trees died after severe defoliation by *Acacicola orphana* and Maddox (1995) provided anecdotal evidence on several hectares of *Melaleuca* plantation which were stripped of all new growth over a one month period in 1992 by *Paropsisterna tigrina*. The 17 % death in trees observed by Elliott (1978) suggests that *A. orphana* could cause substantial economic losses if plantations of this species were established. Research on the insect and its host-plant interactions could result in future management programs for the insect and enable plantations of *A. dealbata* to be successfully grown in Tasmania.

2.9 Ecological theory and studies of *A. orphana*

The information in the preceding sections has shown how little is known about paropsine ecology. Many of the observations and experiments have concentrated on the economically important species and serves to highlight how little is really known about the population and community ecology of even the better known paropsine species. A variety of factors may affect insect population dynamics and have been discussed by numerous authors including Nicholson

(1954), Hairston *et al.* (1960), Root (1973), Dempster and Pollard (1981), Fretwell (1987), Price (1988), Murdoch (1994) and Rodenhouse *et al.* (1997). These authors have all recognised that populations are regulated in some manner, by habitats, hosts or natural enemies. Nicholson (1954 – *"density-dependence theory"*) considered that population regulation occurred via growth in favourable times and reduction through natural mortality and intraspecific competition. Following this idea, Hairston *et al.* (1960) discussed regulation in terms of producers being limited by their own depletion of a resource and hypothesized that a population regulates itself and its own resources. Dempster and Pollard (1981) question this idea, suggesting that few studies have actually quantified the resources available to insects and thus there might be no basis for this assumption. Hunter and Price (1992) further discussed the ideas of top-down, bottom-up limitations on populations and determined that a number of biotic and abiotic factors can regulate populations. The debate over what may regulate populations is still unresolved.

The idea that several abiotic and biotic factors regulate populations is apparent within the literature on paropsines. Outbreaks of *P. atomaria* and *C. bimaculata* occur when climatic conditions are favourable and hosts are available, yet natural predators and parasitoids may also limit populations considerably. The relationship between these regulatory factors and *A. orphana* will be one area covered by this thesis.

3. Basic biology and descriptions of *Acacicola orphana*

Abstract

Basic biological information and figures detailing the different life-stages of *Acacicola orphana* are presented. The insect has a univoltine lifecycle with egg, four larval, pre-pupal, pupal and adult stages. Eggs are laid in a variety of patterns, on the underside of *A. dealbata* and *A. mearnsii* leaves. The different larval stages were found to have head capsule widths ranging from 0.4 to 1.1 mm.

3.1 Introduction

Few authors have conducted detailed taxonomic descriptions of all stages of any paropsine insect, and thus the taxonomy of the paropsines is poorly recorded. Cumpston (1939) discussed the bionomics of seven *Paropsis* species and provided detailed drawings, with emphasis placed on the tubercle positions on the larvae. She observed a lack of variation within species regarding oviposition patterns and larval behaviours, which could be diagnostic. De Little (1979a, b) examined a large number of Tasmanian *Eucalyptus*-feeding paropsines and provided information on these. He presents figures and descriptions of eggs, larvae and adults. These descriptions form a guide to determination of the species in field conditions. Maddox (1995) provided information on the different stages of *Paropsisterna tigrina*, but no other paropsines have been described in detail. Reid (1992) published the first and only pupal key and descriptions. He provides descriptions and detailed line drawings of 9 genera, with classification based on characters including urogomphi, tubercles and setae.

Sexual dimorphism is present within the paropsines (de Little, 1979a; Reid and Ohmart, 1989; Maddox, 1995). Blackburn (1898) noted that mature males were 'wider than the females' however no further information is given regarding the determination of sexual differentiation in *A. orphana* adults. Reid and Ohmart (1989) observed sexual dimorphism in pupae through examination of the sternites, VIII, IX and X. De Little (1979a) determined sex of *C. bimaculata* adults through examination of the proximal tarsal segment of the anterior two pairs of legs which was laterally expanded and rounded in males, compared to females.

Whilst *A. orphana* has been recorded as a severe defoliator of *A. dealbata* and *A. mearnsii* by several authors (French 1911; Froggatt, 1923; Elliott, 1978) little descriptive or biological information has been published on the genus or species (but see Reid, 1992). Before commencing the experiments in the following chapters it was essential to accurately identify the different stages of *A. orphana.* It is proposed that the basic descriptions and biological information obtained will enable future researchers to identify the insect in field conditions and provide a base for formal taxonomic studies in the future. As the taxonomy of the paropsines is currently under review and little is known of the taxonomy or diversity of other *Acacicola* species, no attempt to compare *A. orphana* to other insects in the genus or sub-tribe will be made. To undertake such a task was considered beyond the scope of this thesis.

3.2 Materials and Methods

3.2.1 Determination of adults

Adults were collected from several field locations by beating *A. dealbata* foliage over a beating tray. These were then taken to the laboratory. Initially H. Elliott (Private Forests Tasmania) identified adults. Erichson (1842) and Blackburn (1898) also provide descriptions.

Four methods were used to determine sexual differentiation in adults; separation during copulation, tarsal examination (de Little, 1979a; Stork, 1980), size (length and width measures) and dissection. Adult measurements were made using electronic vernier calipers (limit of measurement 0.1 mm). All adults had been killed and frozen until measured. Significant differences in size were determined using a t-test.

3.2.2 Determination of eggs

To obtain eggs and confirm their identification, adults were caged in the laboratory and provided with excised *A. dealbata* foliage. Foliage was changed daily and eggs removed. These eggs were incubated and larvae were fed regularly until they developed into adults. Eggs were measured using a microscope with a calibrated graticule (limit of measurement = 0.01 mm).

To briefly examine the type of foliage chosen for oviposition and hence where best to search for eggs in the field, a small *A. dealbata* tree (approximately 1.3m) was subjected to a detailed search. Any leaves with eggs were removed and returned to the laboratory where the number of eggs was counted. The leaves were also scored as old or new. Old foliage of *A. dealbata* was simple to differentiate from new foliage, as it was brittle and the pinnules broke off easily. New foliage was more supple and the pinnules did not break off when touched.

3.2.3 Determination of immature stages

Both field and laboratory methods were used to determine larval stages. In the laboratory, larvae were reared from eggs and killed by freezing after emergence or moult to enable measurement. In the field, larvae were collected at fortnightly intervals from three different sites in Tasmania during the period May 14, 1996 to November 7, 1996. The three sites were; Lake Leake (42° 00'S, 147° 47'E), Ridgley (41° 09'S, 145° 48'E) and Arve (43° 09'S, 146° 50'E). Larvae were collected by removing 30 cm lengths from the tips of branches from 15 *A. dealbata* trees at each site. Branches were selected from three heights within the canopy (low, middle and high) and from different aspects. Trees sampled were up to 3m tall. Samples were bagged and returned to the laboratory where larvae were removed from the foliage.

Head capsule widths of all larvae collected were measured using a travelling monocular microscope with a calibrated graticule (graticule divisions = 0.0238mm). Larval lengths were made using a dissecting binocular microscope with a calibrated graticule (graticule divisions = 0.1 mm).

Pupal identification was determined via laboratory rearing. Reid (1992) also provides a key to pupal stages of *Acacicola* (published as *Pyrgoides*).

3.3 Results

3.3.1 Determination of adults

Adults are bright green in colour with brown and white strips on the elytra (Figure 3.1). These colours fade to a dull brown after death. As noted by Erichson (1842) and Blackburn (1898) the elytra have pairs of fine punctures in a linear arrangement.

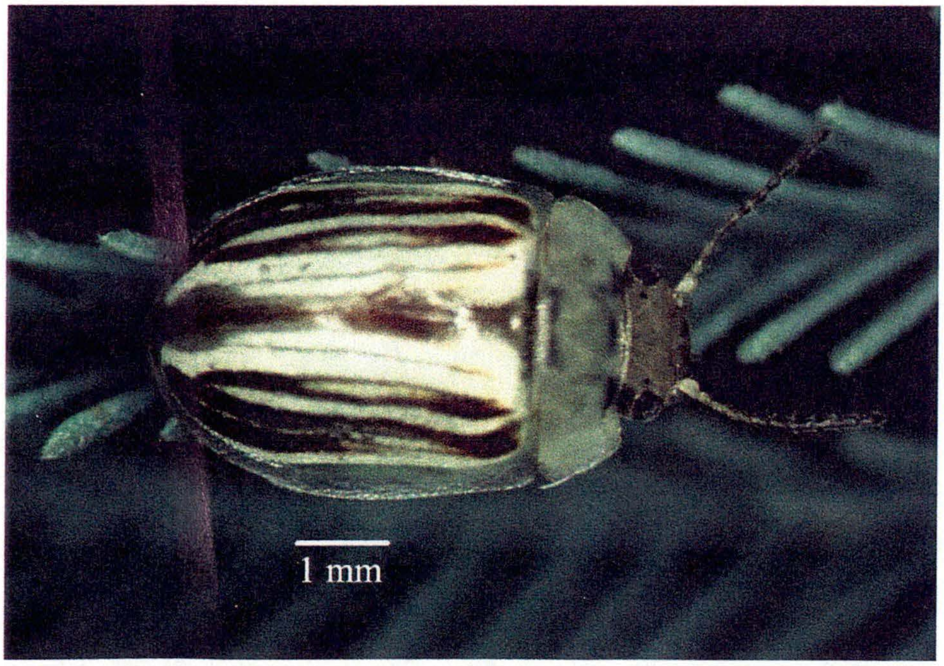


Figure 3.1 – Adult of *A. orphana* on *A. dealbata* foliage

Determining the sex of live *A. orphana* adults was difficult. Adult size measurements (Table 3.1) showed that males were significantly smaller than females (length $t = 6.56$, $df = 155$, $p < 0.001$; width $t = 9.53$, $df = 155$, $p < 0.001$) however, this difference was difficult to detect in the field due to overlap between the sexes, and was therefore not considered to be an acceptable method of sexing live adults.

Table 3.1 - Length and width (± SE) of *A. orphana* adults. (All measurements in mm.)

Sex	Count	Average Length	Average Width	Range (length)	Range (width)
Male	77	5.11 ± 0.039	3.47 ± 0.030	4.1 – 5.9	2.7 – 4.1
Female	80	5.68 ± 0.046	3.77 ± 0.034	4.8 – 6.5	3.1 – 4.8

Attempts to sex adults using tarsal differences were unsuccessful. No differences between male and female tarsi were observed (n=23 pairs). Separation of males and females when in a copulatory position was 92% successful (n=25), although some pairs had both members male. Unfortunately at all times, the most accurate method of determining sex was found to be dissection. However, during March when females were commencing oviposition it was possible to sex adults without dissection with 93% accuracy (n=30), as fecund females had distended abdomens which were light green in colour compared to males which were black in the abdominal region. This method was not accurate for females that were not gravid.

3.3.2 Determination of eggs

Eggs were found on both sides of leaves, usually along the mid-rib. They were 1.38 ± 0.014 mm long, 0.43 ± 0.009 mm wide (mean ± SE; n = 26) and yellow to pale green in colour (Figure 3.2). As the eggs develop, dark pigmentation on the larvae and hatching spines can be observed through the chorion prior to eclosion (Figure 3.3). There was no single pattern of oviposition, as eggs were laid in chains or in clumps on the underside of the leaves, along the midrib and across pinnules. Rarely eggs were also observed on the topside of leaves and on green stem material. Egg batches were found equally on new and old foliage, ($t = 2.02$, $df = 39$, $p = 0.287$). An average of 43 ± 6 (range = 4 – 198, n = 40) eggs were laid on each occupied leaf. In Tasmania, eggs were found on foliage of *A. dealbata* and *A. mearnsii* from March through to July.

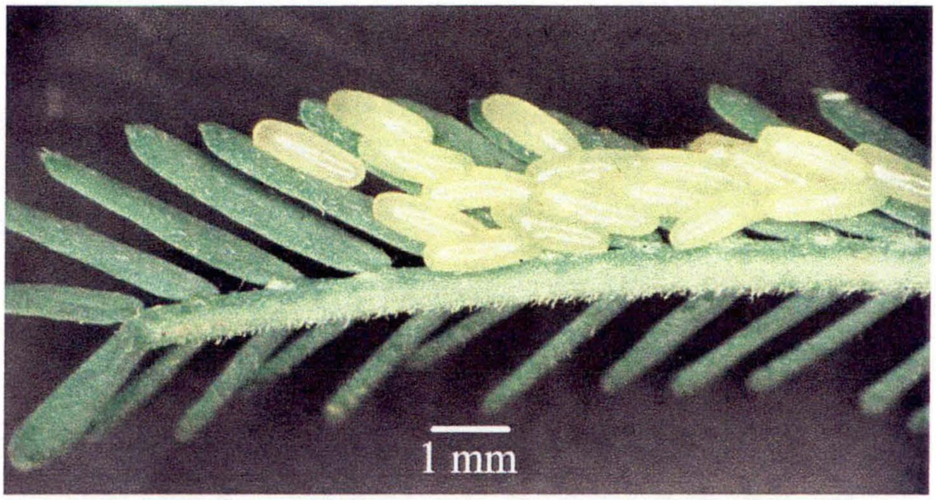


Figure 3.2 - Eggs of *A. orphana* on *A. dealbata* foliage

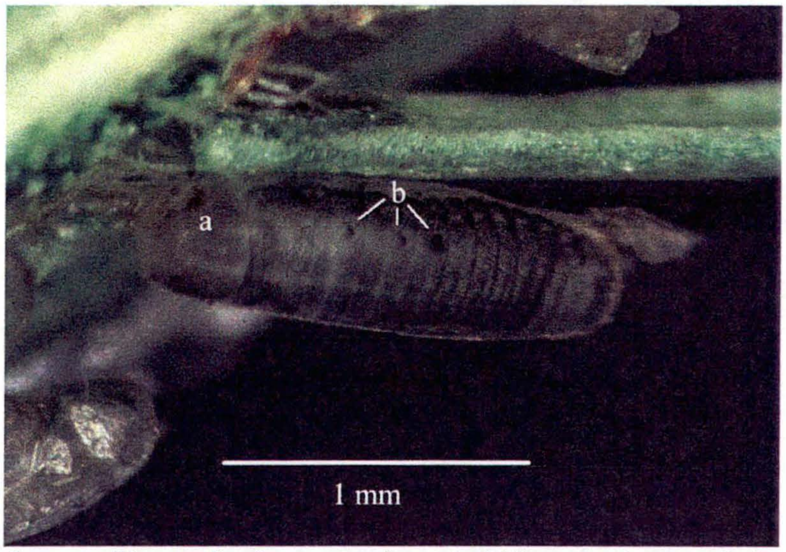


Figure 3.3 - Larva with egg chorion approximately 24 hours prior to eclosion. The head region (a) and hatching spines (b) are marked in the photograph.

3.3.3 Determination of immature stages

A total of 3624 larvae were measured during the 1996 season. Examination of 95% confidence intervals (Genstat 5v3.2) for each larval stage and location showed that the head capsule width of larvae collected from the three different field locations were not significantly different (Table 3.2), and thus results were grouped for the analysis. Using this combined data, four significantly different larval stages were identified using head capsule width measurements (Figure 3.4, Table 3.3).

Table 3.2 - Average head capsule width (SE), count and range of the head capsule width measurements at each of the three field locations for each larval instar.

Stage	Arve Mean ± SE (n) Range	Ridgley Mean ± SE (n) Range	Lake Leake Mean ± SE (n) Range
First instar*	0.45 ± 0.0002[a] (905) 0.36 - 0.52	0.43 ± 0.0003[a] (564) 0.38 – 0.52	0.44 ± 0.0007[a] (203) 0.36 – 0.50
Second instar*	0.60 ± 0.0002[b] (898) 0.55 – 0.69	0.57 ± 0.0007[b] (194) 0.55 – 0.64	0.60 ± 0.0012[b] (135) 0.55 – 0.67
Third instar*	0.83 ± 0.0004[c] (463) 0.76 – 0.95	0.80 ± 0.0038[c] (47) 0.74 – 0.88	0.85 ± 0.0028[c] (68) 0.76 – 0.90
Fourth instar*	1.15 ± 0.0043[d] (50) 1.05 – 1.24	1.09 ± 0.0028[d] (8) 1.05 – 1.19	1.15 ± 0.0023[d] (89) 1.07 – 1.21

*numbers with different letters in the same row are significantly different (95% confidence interval in Genstat 5v3.2).

Table 3.3 – Average head capsule width (± SE) and range of the four larval stages of *A. orphana*.

Stage	Count (n)	Mean ± SE* (mm)	Min. (mm)	Max. (mm)
First instar	1672	0.44 ± 0.00058^{a}	0.29	0.50
Second instar	1227	0.60 ± 0.00075^{b}	0.52	0.69
Third instar	578	0.83 ± 0.00146^{c}	0.74	0.95
Fourth instar	147	1.14 ± 0.00374^{d}	1.02	1.24

*numbers with different letters in the same column are significantly different (95% confidence interval in Genstat 5v3.2).

The reduction in numbers of larvae in the later instars was due to fewer larvae being present in the samples, and may have been an indication of natural mortality. If the number of larvae in each stage is considered as a percentage of the number in the preceding stage, then mortality for the first three instars was 23 %, 53 %, and 75 % respectively. Overall mortality was high, with 91 % of first instar larvae dying by the time they reached the fourth instar.

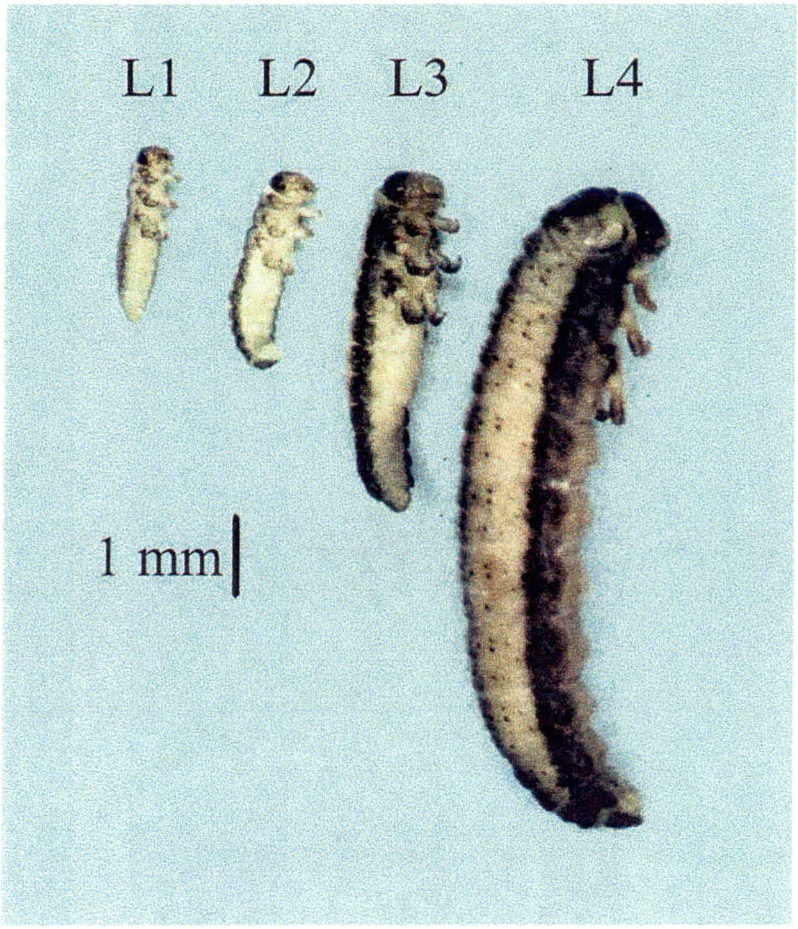


Figure 3.4 – First, second, third and fourth instar larvae of *A. orphana*

The four larval stages are green with setae on the legs and head. Each larval stage has 16 pairs of black tubercles, located on either side of the dorsal mid-line of the abdominal segments. A pair of darkened spots is present on each of the first three abdominal segments and these indicate hatching spines in the first instar. Nine pairs of spiracles are present, one pair are on the thoracic segment and a further 8 pairs on the abdominal segments (Figure 3.5).

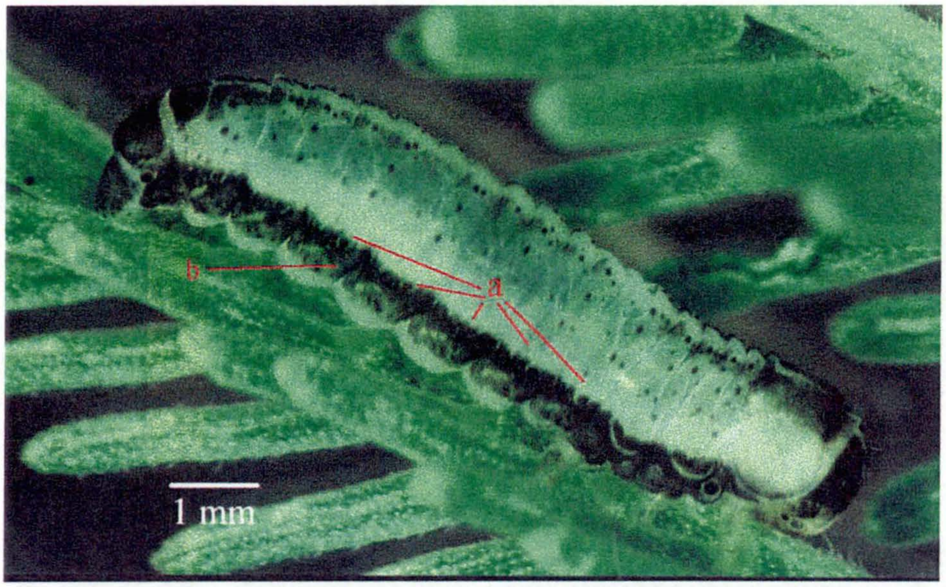


Figure 3.5 – Fourth instar *A. orphana*. Five of the nine spiracles are indicated by (a), the dark pigmented stripe is shown by (b).

Eversible glands are present on the 8th abdominal tergite. These are protruded when the larva is threatened. A dark pigmented longitudinal stripe is present on both sides of larvae of all stages, however it is most apparent in the fourth instar (shown as 'b' Figure 3.5).

Larval lengths vary within stages and are not a reliable indicator of larval stages (Table 3.4). This may be due to a number of factors, including age and diet. All stages overlapped slightly, except the fourth instar.

Table 3.4 – Mean larval body length and range of the four instars of *A. orphana*.

Larval stage	Number	Mean ± SE (mm)	Min. (mm)	Max. (mm)
First instar	20	1.8 ± 0.0919	1.3	2.8
Second instar	20	3.4 ± 0.1190	1.7	3.8
Third instar	20	5.1 ± 0.1324	3.7	6.0
Fourth instar	20	8.9 ± 0.1534	7.3	11.2

After eclosion, larvae were found feeding and resting on both sides of leaves on all sections of the tree, but mainly on terminal shoots. All larval stages can be often found aligned with the pinnules or mid-ribs of the leaves, although later instar larvae are very mobile and can be observed on the bark and green stems of the trees also.

When fourth instar are fully developed, they move down the tree to the soil where they enter a pharate pupal (also known as pre-pupal) stage, enclosed in a small cell in the soil. This stage is similar to the fourth instar except the larvae are paler in colour and do not feed.

Pupating larvae are covered with setae as described in Reid (1992). They are generally yellow in colour (Figure 3.6) but approximately 30% were found to be pale green. The reason for the different colour is unknown, but is most likely related to diet.

Figure 3.6 – Pupa of *A. orphana*.

3.4 Discussion

Acacicola orphana is univoltine. It has an egg stage, four larval stages, pharate pupal, pupal and adult stages which are consistent with other paropsines (Carne, 1966; Greaves, 1966; Styles, 1970; de Little, 1979a; Tribe and Cillie, 1997; Ramsden and Elek, 1998). However, unlike other paropsines that have been studied, *A. orphana* does not lay its eggs in a single diagnostic pattern nor do the larvae exhibit gregarious behaviour (see Chapter 2). The size measurements of the eggs and body lengths of the four larval stages support the observations made during Elliott's 1978 study, where he found eggs to be 1.5 mm long, and larval lengths of 1.68, 3.61, 5.00 and 8.69 mm for each of the four stages. The head capsule widths recorded by Elliott (1978) were also similar to those observed in this study (0.22, 0.57, 0.79 and 1.15 m respectively), except in the first instar. For this stage, Elliott recorded 0.22 mm (range 0.18 - 0.24 mm) which is small compared to the 0.44 mm (range: 0.29-0.50 mm) recorded for first instar *A. orphana* larvae here. Elliott (1978) does not note whether his larvae are laboratory or field reared or whether larvae were randomly selected for measurement, but any of these variables may have affected his result.

Sexual dimorphism through examination of tarsi was not observed in this species, although abdominal colour and size can be used to differentiate males and fecund females prior to oviposition with reasonable accuracy. Examination of more *Acacicola* species may provide further insight into whether or not any sexual dimorphism exists within this genus.

It is proposed that when the new key to the Paropsina is published it will provide detailed couplets to enable determination of the adult from taxonomic characters (C.A.M. Reid, pers. comm.). A detailed taxonomic study of *Acacicola* may also clarify the issue of sexual dimorphism. However, the working guide within this chapter provides enough information for researchers to identify the insect in the field.

4. Development of *A. orphana* in field and laboratory studies.

Abstract

Developmental biology of *A. orphana* was examined in the laboratory at constant temperatures. Day-degree developmental times and minimum temperature thresholds were determined for each stage. Development was also studied at three field sites, over a period of 22 months. Predictions of developmental estimates in the field were made based on the laboratory model, and compared to the observed development at the three sites. This comparison showed the laboratory model did not accurately predict development in the field and reasons for this are presented. Longevity, sex ratios, mating and reproductive behaviour of *A. orphana* are also examined.

4.1 Introduction

4.1.1 Modelling insect development

Detailed knowledge of the lifecycle and development rate of pest insects is important to reduce economic losses caused by unexpected outbreaks or unnecessary chemical applications. For several decades chemical sprays have been the main method of insect control, but environmental considerations and pesticide resistance concerns have resulted in a shift to more 'environmentally friendly' methods including biocides, natural predators and parasitoids. One limitation of these methods of pest control however, is that they often target specific stages of the pest. It is therefore essential to know the developmental times of each stage so that control can be applied with maximum efficacy (Harcourt, 1969; Stedinger and Shoemaker, 1985; Hudes and Shoemaker, 1988). Development studies also enable estimation of maximum and minimum temperature thresholds and these can help to characterize the biology and geographic distribution of an insect.

Development is the change from fertilised egg to adult (Torre-Bueno, 1989). The rate of development is the reciprocal of time (Howe, 1967; Campbell *et al.*, 1974) and can be affected by several factors including relative humidity, food type, photoperiod and light intensity. Insect behaviour such as basking may also influence development (Maddox, 1995; Lactin *et al.*, 1995; Clarke, 1998).

When determining which developmental model to use it is necessary to consider where it will be used and what variables may affect its accuracy. Many of the variables affecting development can be maintained at a relatively constant level in a laboratory. However, a model developed in constant temperature conditions may not be accurate when transferred to the field as insects exposed to variable conditions develop faster (Rock, 1985; Regniere and Bolstad, 1994). Inconsistencies in developmental data between field and laboratory conditions have been shown in studies on the Colorado potato beetle, *Leptinotarsa decemlineata* (Tauber *et al.*, 1988a; Tauber *et al.*, 1988b) and the Carrot fly, *Psila rosae* (Fabricius) (Collier and Finch, 1996), suggesting that a model which is to be used in the field should be created with field data and *vice versa*. In some instances however, field and laboratory data have been fitted successfully to the same model (Got *et al.*, 1996).

Published models for predicting development in both field and laboratory conditions have been linear (Hughes, 1963; Campbell *et al.*, 1974; Osawa *et al.*, 1983) and non-linear (Logan *et al.*, 1976; Taylor and Harcourt, 1978; Hudes and Shoemaker, 1988). The relationship between insect development rate and temperature is of the form shown in Figure 4.1.

Linear models utilise the mid-range developmental temperatures (shown as the area between the two vertical lines in Figure 4.1). These models do not consider upper and lower extremes in temperature as these parts of the relationship are non-linear (Campbell *et al.*, 1974). When using a linear model, the minimum temperature threshold (T_o) can be estimated directly from extrapolation of the regression line to the x-axis and day-degrees can be calculated as the reciprocal of the slope. Alternatively, non-linear models are more accurate when temperature extremes are encountered in the field but concentrate on proportional developmental values per unit time rather than day-degree measurements (Kitching, 1977).

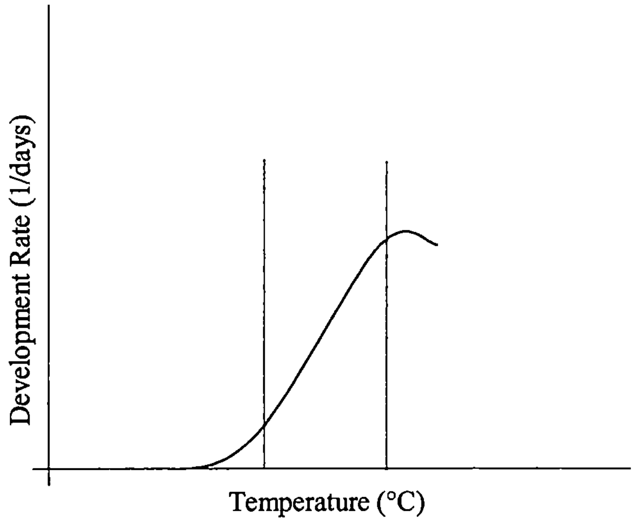


Figure 4.1 - Relationship of developmental rate with temperature (heavy line). The space between the finer vertical lines indicates the linear portion of the curve.

4.1.2 Development of insects in the sub-tribe Paropsini

Several authors have examined the development of paropsine species including *P. charybdis* (Styles, 1970), *Ps. tigrina* (Maddox, 1995), *P. atomaria* (Carne, 1966a), *C. bimaculata* (Clarke, 1998), *C. agricola* (Ramsden and Elek, 1998) and *T. tincticollis* (Tribe and Cillie, 1997). They have shown that the paropsine lifecycle consists of eggs, four larval stages, pre-pupa, pupa and adults. Day-degree (DD) developmental times for many of the species including *A. orphana* are lacking in the literature, and thus it is difficult to make comparisons of development rates between different species. Published developmental times for three species are shown in Table 4.1.

Table 4.1 - Estimates of developmental times in day-degrees for three paropsine species; *C. bimaculata*, *P. atomaria* and *Ps. tigrina*.

Species	Egg	First instar	Second instar	Third instar	Fourth instar	Pre-pupa + pupae
C. bimaculata[1]	112	49	41	46	64	184
P. atomaria[2]	182	101	68	67	65	306
Ps. tigrina[3]	57	32	26	27	50	92

[1]Clarke (1998)
[2]Calculated from Carne (1966a).
[3]Maddox (1995)

There is considerable variation between the physiological developmental times of the different paropsine species in Table 4.1. Insect behaviour such as basking or sheltering may affect the rate of development and cause inaccuracies in developmental estimates based on air temperature alone. For example, the Colorado potato beetle can increase body temperature above air temperature by basking (Lactin *et al.*, 1995). Maddox (1995) noted that *Ps. tigrina* could increase body temperature by more than 8 °C above ambient temperature by basking, which can resulting in inaccuracies in developmental time estimates of up to 45%. Minimum temperature thresholds for development also vary between species, with Clarke (1998) reporting an average of 3.95 °C (range: 0.55 - 5.50 °C) for *C. bimaculata* which is considerably less than the average value of 11.6 °C for *Ps. tigrina* (Maddox, 1995). Re-analysis of data presented by Carne (1966a) suggested that *P. atomaria* has an average minimum temperature threshold of 6.3 °C (range 3.7 – 9.2 °C).

4.1.3 Chapter outline and aims

Basic biological and developmental information is essential prior to developing a management program for any pest species. No author has conducted detailed studies of aspects of the lifecycle of *A. orphana*. Elliott (1978) noted *A. orphana* larvae develop during the winter months, which is in contrast to the summer lifecycle of other paropsines. Development at a time of year that is unfavourable for many invertebrate natural enemies may mean there are few natural population control methods for *A. orphana*, as suggested by Elliott (1978) (see Chapter 5). Once detailed lifecycle information is known, it is possible to examine the management options available and build a management program based on accurate information, rather than assumptions. For accurate developmental estimates, it was decided that field and laboratory methods would be used, and the results compared. Detailed knowledge of the development of *A. orphana* in respect to temperature would also provide a base of information on the lifecycle and potential geographical distribution of this pest should its hosts, *A. dealbata* or *A. mearnsii* become a plantation species in southeastern Australia.

With these considerations in mind, the following aims were identified for this chapter;

1. To examine the development of each insect stage in the laboratory and obtain estimates of minimum temperature thresholds and developmental times.
2. To study the phenology of the insect in field conditions and compare field and laboratory developmental information.
3. To examine other aspects of the biology of *A. orphana* including adult longevity and reproduction.

4.2 Materials and Methods

4.2.1 Developmental studies and adult longevity at constant temperatures

4.2.1.1 Development

During the months of April 1996 and April 1997, mature adults were collected from the field and caged in the laboratory with either excised *Acacia dealbata* foliage which was changed daily or small potted *A. dealbata* trees. Foliage was checked daily for eggs. Leaves with eggs were removed and individual leaflets supporting eggs were randomly placed in 9 cm plastic Petri plates that were then enclosed in larger plastic containers. Whilst it was not possible to randomly allocate individual eggs to treatments, each dish contained eggs from a minimum of three different leaves. Wet filter paper was placed in the base to avoid desiccation. The containers were then incubated across constant temperatures of 6, 8, 14, 17, 22 and 25 °C, all ± 1°C in Contherm CAT 150 MCP cooled incubators. Relative humidity was approximately 66% except in the 25°C treatment, where it was 45%. All incubators had a photoperiod of 8L:16D hours. Incubator lighting was provided by two 40W fluorescent tubes, emitting light of intensity 220 ± 20 lux. Using the same method, more cultures were incubated at temperatures of 12, 15, 18, 20 and 23°C ± 1 °C in April 1999.

Over the three seasons, the number of eggs incubated at each temperature were as follows;

6 °C	- 3 containers, each with 21, 23 & 24 eggs	(total = 68 eggs)
8 °C	- 6 containers, each with 20, 26, 21, 20, 20 & 23 eggs	(total = 130 eggs)
12 °C	- 4 containers, each with 20, 23, 22 & 21 eggs	(total = 86 eggs)
14 °C	- 7 containers, each with 23, 21, 20, 20, 22, 19 & 27 eggs	(total = 152 eggs)
15 °C	- 4 containers, each with 16, 20, 19 & 18 eggs	(total = 73 eggs)
17 °C	- 5 containers, each with 21, 26, 21, 17 & 22 eggs	(total = 111 eggs)
18 °C	- 4 containers, each with 19, 19, 20 & 22 eggs	(total = 80 eggs)
20 °C	- 6 containers, each with 25, 25, 18, 22, 15 & 23 eggs	(total = 128 eggs)
22 °C	- 9 containers, each with 35, 21, 27, 20, 23, 16, 22, 21 & 18 eggs	(total = 227 eggs)
23 °C	- 4 containers, each with 17, 22, 16 & 13 eggs	(total = 68 eggs)
25 °C	- 2 containers, each with 26 & 22 eggs	(total = 48 eggs)

Due to high mortality during the first two seasons, more cultures were incubated in the worst affected temperatures when eggs became available. During 1999, more cultures were incubated to improve the accuracy of the results, but were not followed through to the adult stage, due to time constraints.

After eclosion, larvae were fed an abundant amount of foliage sourced from a single *A. dealbata* tree. A single tree was chosen because it was known that larvae could feed and survive on the foliage of this particular tree. It was also considered that use of foliage from a single source would eliminate variation in development time that could be caused by foliage of varying quality from different trees. The foliage was changed three times weekly except in the 25°C treatment where it was changed daily.

The 6 °C and 8 °C cultures were checked three times weekly for development. Cultures at all other temperatures were inspected for development daily. At each inspection, numbers of larvae in each stage were counted. Average development time in days and mortality were recorded for each stage.

Day-degrees (DD) and minimum temperature thresholds (T_o) were calculated from a graph of mean development rate (*y*, reciprocal of developmental time in days) versus temperature (T, °C) for each cohort (container) of insects. The resulting regression equation (Equation 1), where *a* and *b* are constants, allows calculation of the minimum temperature threshold (T_o) using Equation 2, and the day-degree developmental time (DD) (Equation 3).

$$y = ax + b \qquad \text{Equation 1}$$

$$T_o = \frac{-b}{a} \qquad \text{Equation 2}$$

$$\text{DD} = \frac{1}{a} \qquad \text{Equation 3}$$

The standard error of the minimum temperature threshold and the day-degree development time estimate was calculated using Equations 4 and 5, by Campbell *et al.* (1974).

$$\text{SE of } T_0 = \frac{y}{b}\sqrt{\frac{s^2}{N\bar{y}^2} + \left[\frac{\text{S.E. of } b}{b}\right]^2} \qquad \text{Equation 4}$$

$$\text{SE of DD} = \frac{S.E.of\, b}{b^2} \qquad \text{Equation 5}$$

Where $\bar{y}$ is the mean developmental time (in days) of the sample and s^2 is variance of y, the development rate. The slope of the linear regression is b and N is the number of values.

4.2.1.2 Adult longevity

Fourth instar larvae were collected from the field and cohorts of 20 individuals were incubated as described in Section 4.2.1.1. All cultures were cleaned and fresh excised *A. dealbata* foliage was provided three times weekly except in the 25°C culture, where it was changed daily. Time from emergence as adults until death was recorded. Analysis of variance was used to determine the effect of different temperatures on longevity. The sex of the adults was not determined.

4.2.2 Development studies in the field

Three sites in Tasmania were monitored at fortnightly intervals from April 22, 1996 to June 18, 1997 (1996 season) and approximately monthly from June 18, 1997 through to March 28, 1998 (1997 season). The three sites were over 100km apart and experience different climatic conditions. Very little was known of the insects' biology and so three sites were chosen to obtain a wider picture of the population phenology and to guarantee that at least one population would survive the duration of the study. Using three sites would also enable developmental estimates to be calculated using a linear model. Site locations are shown in Figure 4.2 and descriptions are as follows;

Site 1 - Lake Leake

Lake Leake is situated 30 km east of Campbelltown (42° 00'S, 147° 47'E). The sample site was at an elevation of approximately 580m ASL and sloped gently downwards to the southern edge of the lake. Vegetation on the site was predominantly native grasses and *A. dealbata* trees. Approximately 15 years prior to sampling, the site was severely burned by a bushfire which may have caused the subsequent germination of the *A. dealbata* trees that were sampled at the site. The trees were small, generally 1-3 m in height, although some trees up to 5 m were present. Many of the trees were stunted and bushy in appearance. The area surveyed was approximately 50 x 50 m. This site had the lowest mean annual temperature and rainfall of the three sites examined (Table 4.2).

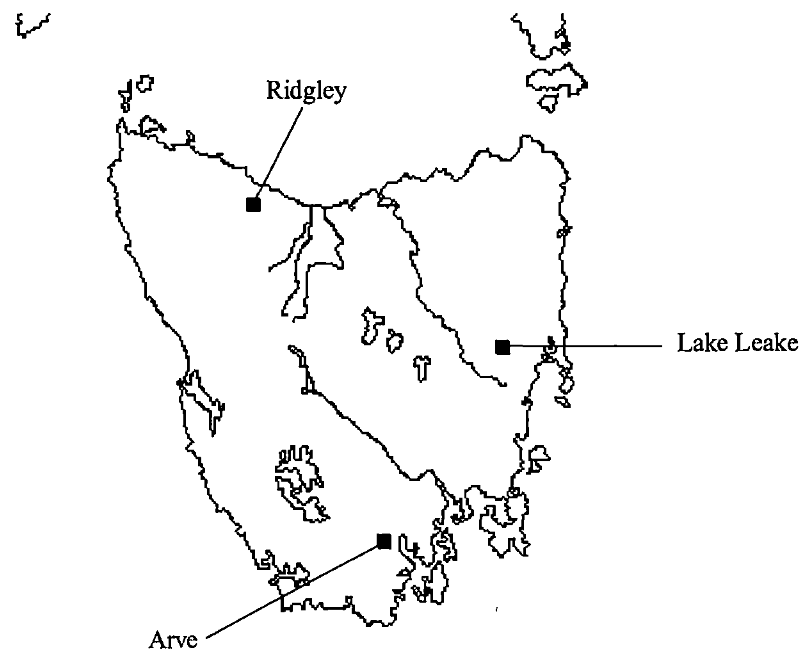


Figure 4.2 - Location of the three sites used to determine field developmental data in Tasmania.

Site 2 - Arve

The Arve sample site was located approximately 280m ASL in the Southern Forests (43°09'S, 146°50'E), approximately eight kilometres west of Geeveston in southern Tasmania. The *A. dealbata* trees sampled were growing randomly in a *Eucalyptus* plantation. The area surveyed was approximately 50 m x 20 m and included trees lining the sides of a fire track. Most trees were 1-7 m tall, although branches were sampled from the smaller trees, to enable sampling of the whole tree. This site had only slightly higher annual rainfall than the Lake Leake site and the highest mean annual temperature of the three sites (Table 4.2). The *A. dealbata* trees at this site had tall straight stems and appeared very healthy. This may be due to a combination of competition for light from the *Eucalyptus* trees and the climatic conditions.

Table 4.2 - Climatic data for Lake Leake, Arve and Ridgley.

Climatic Variable	Lake Leake[1]	Arve[2]	Ridgley[3]
Total average rainfall (mm)	850	880	1254
Average number of rain days per year	130	165	136
Highest mean monthly rainfall (mm)	90.4	97.6	170.9
Lowest mean monthly rainfall (mm)	45.7	40.5	52.4
Mean annual temperature (°C)	8.9	11.6	9.7
Mean max. temperature of the warmest month (°C)	19.9	21.9	18.9
Mean min. temperature of the coolest month (°C)	0.3	1.3	3.0

[1]Averages for rainfall based on 104 years of data. Temperature averages are based on data accumulated over 27 years.
[2]Averages of rainfall based on 84 years of data. Temperature averages based on data accumulated over 26 years.
[3]Averages for rainfall based on 23 years of data. Temperature averages are from Tewkesbury, approx. 7 km from Ridgley and are based on 64 years of data.
(All data courtesy of the Bureau of Meteorology, Climate and Consultancy Section, Tasmania, 1996.)

Site 3 - Ridgley

This site was situated on the side of a small valley at Ridgley in North-west Tasmania (41°10"S, 145° 48"E, 280m ASL) and was part of a well maintained reserve surrounded on one side by *Eucalyptus* plantations and on the remaining sides by farmland. A small river flowed along the western boundary. This site had the highest mean annual rainfall of the three sites examined (Table 4.2). The average annual temperature was mid-way between the other two sites, but the average lowest temperature was higher than observed for both Lake Leake and Arve (Table 4.2). This may be due in part, to the close proximity of this site to the Tasmanian coast. The area sampled at this site was approximately 100 m x 50 m. A variety of tree species were present in the study area and apart from *A. dealbata,* common trees were *A. melanoxlylon* and *Eucalyptus* species. The trees ranged greatly in age, although sampling was confined to younger trees, up to approximately 3m tall. Thick scrub was present on the boundary of the site adjacent to the *Eucalypytus* plantation and several smaller *A. dealbata* trees grew in this area during the study. The trees at the site were very healthy in appearance with tall straight stems.

4.2.2.1 Sampling method

Samples consisted of 15 x 30cm branch lengths removed from the terminal foliage of a minimum of 10 *A. dealbata* trees from each site on each sample occasion. Wherever possible, the same tree was not sampled twice on the same day. Trees sampled were within reach of the person sampling so branches could be removed at random from high, mid-way and low in the canopy without the use of a ladder. Branches high in the canopy were from as high up in the canopy as could be reached, but were never the main terminal shoots. In the 'low' position the branches taken may have been shaded by the higher branches, as they were in the lower third of the canopy, and branches taken from 'mid-way' were cut from the outer edges of the middle third of the canopy. Different aspects were also sampled to reduce site effects. Samples were returned to the laboratory where they were frozen (-20 °C). Due to the small size of *A. orphana*, a magnification lamp was then used to search the foliage for eggs and other stages. The numbers of insects collected from each stage were counted and larval instars determined by examination of head capsule width under a travelling monocular microscope with a calibrated graticule (Chapter 3).

Before commencing the study, information obtained from the Bureau of Meteorology showed weather stations close to all field sites. Some months into the study it was found that climate data from these stations was not entirely complete, and full climatic data was not available for the Ridgley site. Temperature data for Ridgley was obtained from Dr. Greg Holz of North Eucalypt Technologies for the period April 1996 through to December 1996. After this period Tinytalk II data loggers (Gemini Data Loggers, UK) were installed at all sites. These recorded temperature on a half-hourly basis. Where data was missing for individual days, it was replaced by Bureau of Meteorology long-term temperature averages.

4.2.2.2 Analyses

<u>Ascertaining the peak of each stage in the field</u>

Before using any model to estimate the rate of development, it was necessary to determine the peak of each stage in the field. To do this, the number of insects collected on each sample date was converted into proportions of the total number of insects collected in each stage for each season. These values were then plotted against time (in days) from March 1. As a starting point, March 1 was selected to make the calculations simpler. It was also selected because in the first season it was not known exactly when the eggs had reached a peak in the field. A polynomial regression was fitted to the data, and the peak in insect numbers was calculated (by solving the equation) as the turning point of this (denoted as x on Figure 4.3). This method assumes a normal distribution for each of the individual developmental stages.

Once the number of days to the peak of each stage was determined, it was possible to calculate the duration (in days) for each stage by subtracting one stage from the following stage. Thus, to calculate the duration of the L1 stage, the days to the peak of the L1 stage were subtracted from the days to the peak of the L2 stage. It was then possible to model the data and obtain an estimate of development time for each stage in degree-days, using the two linear methods outlined following.

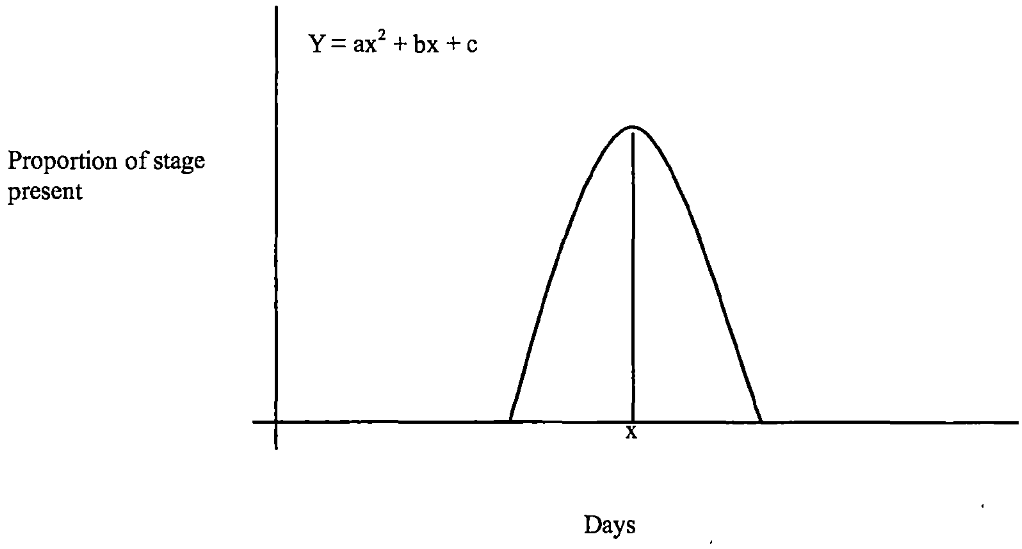


Figure 4.3 – Representation of a polynomial function showing the proportion of insects of a specific stage and development time.

Method 1 - Calculating minimum development thresholds and day-degree estimates for each stage from the field data using a simple linear model.

Once the number of days was known for each stage, the rate of development for the period was calculated (= 1/days). The average temperature for the period (peak to peak) was also calculated from the field-collected temperature data. This was conducted for both seasons at the three sites, giving a total of 6 points (3 sites x 2 seasons) for each linear regression, for each stage. The minimum temperature threshold and an estimate of the day-degree duration of each stage were calculated from these graphs. Standard errors were calculated using Equations 4 and 5. This method allows direct comparison with developmental estimates and minimum temperature thresholds calculated from the laboratory data.

Method 2 - Developmental time in day-degrees of each stage using stage peak observations in the field and the average minimum temperature threshold determined in laboratory studies.

The developmental time for each stage was calculated from the peak of one stage to the next from the field data as outlined previously. A minimum temperature threshold (T_o) of 4.38 °C was used to calculate field development day-degree (DD) estimates, as this was the value obtained for the total egg-adult development in the laboratory study. During the 1996 season only daily maximum (T_1) and minimum (T_2) temperatures were known and Equation 6 was used to calculate average daily temperatures (T_d). These were then summed for the development time of each stage. Cumulative day-degrees for stage *i* (DD_i, *i* = eggs - adult) were calculated as the sum of daily T_d values up to the time of maximum abundance as determined from the polynomial (see x, Figure 4.3) using Equation 7.

$$T_d = \frac{T_1 + T_2}{2} - T_0 \qquad \text{Equation 6}$$

$$DD_i = \sum_{n=1}^{x} T_{d_n} \qquad \text{Equation 7}$$

As the DD values calculated were cumulative, stage specific developmental estimates were determined by subtraction. Data loggers installed in the second season of the experiment provided half-hourly temperature data. Analysis of a subsample of temperature data showed that T_d determined from this data was not significantly different to T_d obtained from averaging daily minimum and maximum temperature data (t = 2.03, df = 34, p = 0.11). In the second season Equation 8 was used to determine T_d.

$$T_d = \Sigma(\ T_t - T_o\ ) \qquad \text{Equation 8}$$

Where T_t is the half-hourly temperature reading.

Two constraints were applied to Equations 6 and 8, these being that if $T_d < 0$ then $T_d = 0$ and if $T_d > 25$ then $T_d = 25$ °C. These constraints are necessary as temperatures below zero negatively affect the summation process and result in underestimation of the day-degrees for development. Hot temperatures may also cause the insect to be stressed and therefore development will be inhibited (Kemp *et al.*, 1986; Ro *et al.*, 1998). The upper threshold value for *A. orphana* was estimated from the laboratory work with the assumption that insects in the field are able to tolerate higher slightly higher temperatures than in the laboratory constant temperature experiments due to diurnal temperature fluctuations.

4.2.2.3 Comparing field and laboratory estimates for development

Laboratory T_0 and DD estimates were directly comparable with the results for the field data using method 1.

The second method of validation for the field model involved combining the estimated laboratory DD for the stage and the field temperature data to give a prediction based on the laboratory DD estimate of what date the stage would be present in the field. The predicted date was then compared to the observed date.

4.2.3 Reproduction - Fecundity

Adults were collected from Buckland (42° 39'S, 147° 36'E) on March 9, 1997. This date was after emergence from the summer resting period and prior to oviposition. A sub-sample was dissected to confirm the assumption that no ovarian development had occurred, although it was not determined if they had already mated in the field. Insects were put into two treatments. Treatment 1 involved 20 pairs of mating insects incubated as pairs to examine the number of eggs a female could lay when continually exposed to a male. Treatment 2 consisted of 34 individual females separated from males immediately post-copulation, to examine how many eggs could be laid after a single laboratory copulation event. This trial would test for differences in egg number and egg fertility over time.

Insects were placed in 9 x 1 cm round plastic Petri dishes lined with moistened filter paper. These were then enclosed in a cylindrical plastic bag and incubated at 17 ± 1 °C. Photoperiod, light intensity and relative humidity were as noted in Section 4.2.2.1. These incubator conditions were found to be optimal for survival during the laboratory constant temperature development studies in section 4.1.

Containers were cleaned and fresh *A. dealbata* foliage was provided three times weekly. At the time of cleaning any eggs laid were collected and incubated separately to assess fertility. The maximum length (from head to rear end of elytra) and maximum breadth (across the elytra) of any dead insects were measured using vernier calipers (accuracy = 0.1mm) to examine the relationship between size and fecundity. If males in treatment 1 died they were replaced until the female died.

4.2.4 Reproduction – sex ratios, mating and ovarian development

To examine ovarian development and sex ratios, *A. orphana* adults were collected from October 1997 until June 1998 at a site near Buckland in southern Tasmania (42° 39'S, 147° 36'E). The site was on a northeastern facing hillside where *A. dealbata* was the predominant species. Trees ranged in size from less than 1m to approximately 10 m. At fortnightly intervals insects were collected by walking through the area and beating foliage over a tray for 30 minutes. After collection the insects were returned to the laboratory where they were killed by freezing, measured with vernier calipers and dissected. Sex ratios were determined and ovarian development was scored on three levels; no development, semi-formed eggs and fully formed eggs.

To examine mating behaviour in the field and determine if females were selective in their choice of mate size or if males were competing for females, single and mating adults were collected from Conara on April 22, 1999. These were labelled as either pairs (if mating) or singles and taken to the laboratory where they were killed and their width and breadth measured using vernier calipers as outlined in section 4.2.3.

4.3 Results

4.3.1 Development and adult longevity at constant temperatures

A total of 1171 eggs were incubated at 11 different temperatures ranging from 6 - 25 °C over three seasons. High mortality was experienced at all temperatures. Although eggs hatched in the 6 and 25 °C treatments, the larvae died before their first moult, suggesting these temperatures are close to the minimum and maximum temperature thresholds of *A. orphana.*

Egg mortality varied with temperature. The 17 °C treatment recorded the lowest egg mortality at 4.5% whilst the 20 and 22 °C treatments both experienced approximately 50% mortality (Table 4.3). Mortality during the first instar accounted for the majority of death overall (76 %), with larvae only surviving this stage in the mid-range (12 – 23 °C) temperature treatments. Stage specific mortality decreased as the larvae developed (Table 4.3) and relatively few deaths were recorded from the fourth instar stage onwards. Pupal mortality at 20 and 22 °C may be attributed to insects being exposed to constant temperatures that were higher than experienced in the field, where these stages are present in the soil. Many of the pupa that did develop into adults at these temperatures were deformed. These results suggest that 14-17 °C was preferable for survival and development of *A. orphana.*

4.3.1.1 Development

Of the 1171 eggs incubated, 856 developed into first instar larvae. Developmental time in days and the number of insects of each stage (in brackets) are shown in Table 4.4. All insects were reared from eggs less than 24 hours old. Attempts to incubate field-collected larvae of different stages were largely unsuccessful, necessitating the use of larvae reared through from eggs. Due to this, results were not obtained for the later stages of treatments at temperatures of 6, 8 or 25 °C.

Larvae developed faster as temperature increased, except at the highest treatment (25 °C) where development was slightly slower than at 23 °C. The development time of first instars at 23 °C was slower than at 22 °C and hence the data for this temperature was also not included in the linear regressions used to determine minimum temperature thresholds and development rates.

Minimum temperature thresholds and day degree development times for the different stages were calculated from the regression lines shown in Figure 4.4.

Table 4.3 - Mortality of *A. orphana* as a percentage of insects developing to that stage and number of insects.

Temperature (°C)	Eggs incubated (n)	Egg (%) (n)	First instar (%) (n)	Second instar (%) (n)	Third instar (%) (n)	Fourth instar (%) (n)	Pre-pupa (%) (n)	Pupa (%) (n)	Total (%) (n)
6	68	8.8 (62)	100						100 (0)
8	130	19.2 (105)	100						100 (0)
12	86	22.1 (67)	80.6 (13)	N/C					-
14	152	13.2 (132)	53.8 (61)	29.5 (43)	30.2 (30)	6.7 (28)	0.0 (28)	0.0 (28)	81.6 (28)
15	73	16.4 (61)	72.1 (17)	N/C					-
17	111	4.5 (106)	45.3 (58)	25.8 (43)	37.2 (27)	7.4 (25)	16.0 (21)	0.0 (21)	81.1 (21)
18	80	27.5 (58)	70.7 (17)	76.5 (4)	N/C				-
20	128	49.2 (65)	46.2 (35)	54.3 (16)	43.8 (9)	11.1 (8)	0.0 (8)	25.0 (6)	95.3 (6)
22	227	52.4 (108)	81.5 (20)	35.0 (13)	23.1 (10)	10.0 (9)	0.0 (9)	22.2 (7)	96.9 (7)
23	68	26.5 (56)	80 (16)	70 (3)	66.7 (1)	N/C			-
25	48	12.5 (42)	100						100 (0)

Note: N/C indicates culture removed from incubator before development completed.

Table 4.4 - Mean developmental time in days (±SE) and number of insects (in brackets) for each temperature and stage.

Temperature (°C)	Eggs	First instar	Second instar	Stage Third instar	Fourth instar	Pre-pupa	Pupa	Total days from Egg-Adult
6	46.7 ± 0.03 (62)	N/R						
8	31.1 ± 2.62 (105)	48.9 ± 0 (2)	N/R					
12	16.0 ± 0.21 (67)	26.3 ± 0.44 (13)	N/C					
14	14.0 ± 0.24 (132)	20.6 ± 0.26 (61)	27.6 ± 0.28 (43)	17.5 ± 0.32 (30)	22.4 ± 0.30 (28)	11.9 ± 0.21 (28)	18.0 ± 0.19 (28)	131.9 ± 0.43 (28)
15	13.0 ± 0.25 (61)	19.2 ± 0.53 (17)	N/C					
17	11.0 ± 0.37 (106)	17.0 ± 0.35 (58)	21.6 ± 0.45 (43)	14.0 ± 0.47 (27)	19.4 ± 0.37 (25)	10.6 ± 0.41 (21)	14.0 ± 0.33 (21)	107.7 ± 0.53 (21)
18	10.0 ± 0.21 (58)	14.84 ± 0.34 (17)	N/C					
20	8.4 ± 0.18 (65)	12.5 ± 0.20 (35)	17.9 ± 0.18 (16)	10.6 ± 0.25 (9)	15.5 ± 0.35 (8)	8.1 ± 0.29 (8)	10.7 ± 0.23 (6)	83.8 ± 0.68 (6)
22	7.6 ± 0.21 (108)	10.3 ± 0.22 (20)	14.9 ± 0.39 (13)	8.0 ± 0.30 (10)	11.9 ± 0.26 (9)	6.1 ± 0.29 (9)	10.0 ± 0 (7)	69.0 ± 1.02 (7)
23	7.0 ± 0.25 (50)	13.0 ± 0.25 (10)	13.0 ± 0 (3)	N/R				
25	7.35 ± 0.04 (26)	N/R	N/R	N/R				

N/R indicates no result due to mortality; N/C indicates culture removed from incubator before development completed.

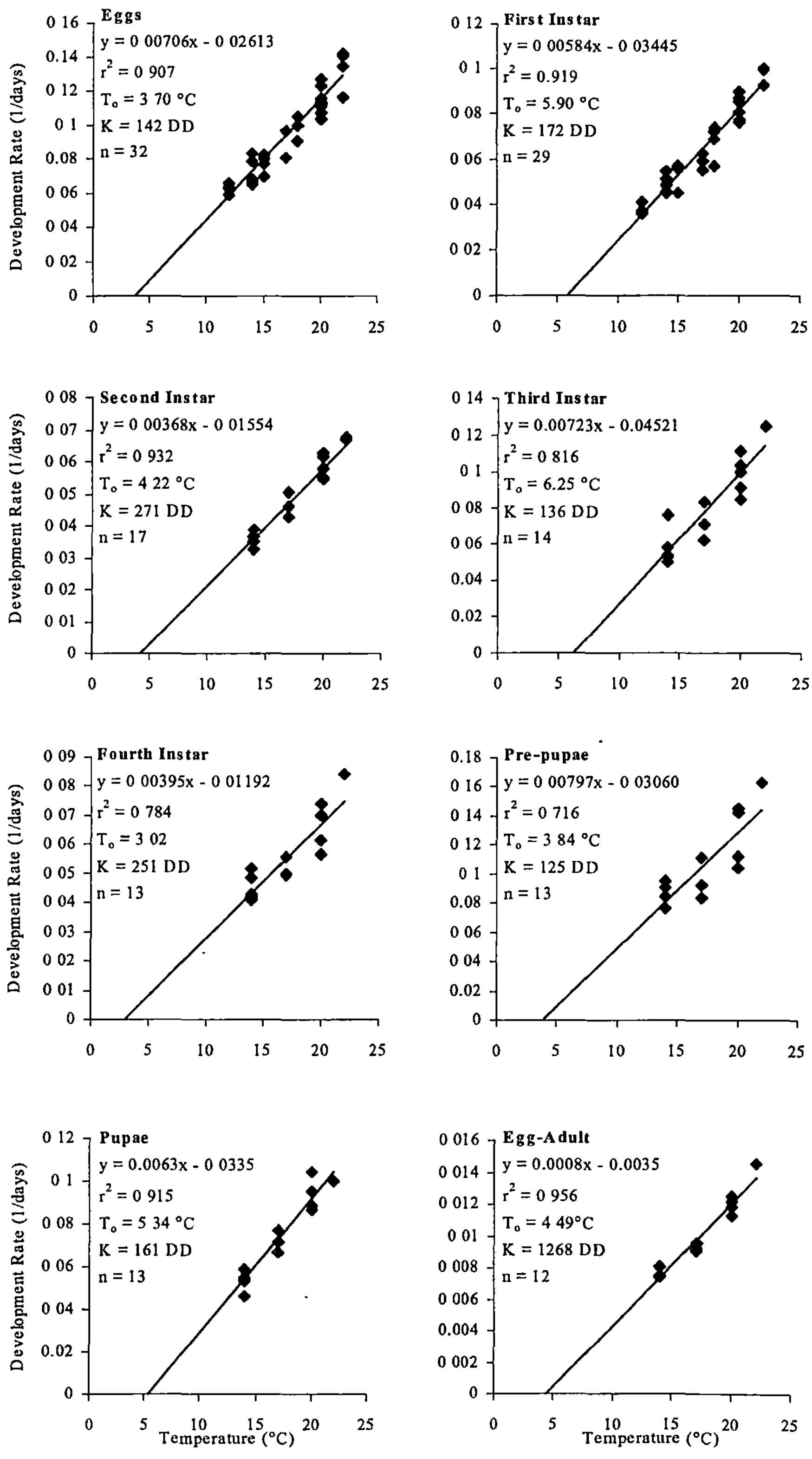


Figure 4.4 - Constant temperature linear models of development rate versus temperature for all stages of *A. orphana*. Regression equation, r^2, developmental estimate and minimum temperature threshold values given. p < 0.001 for all stages.

Table 4.5 – Summary of day-degree development times, minimum temperature thresholds and their standard errors for *A. orphana* life stages based on laboratory constant temperature data.

Stage	Minimum temperature threshold (T_o) °C ± SE*	Development time (DD) ± SE*
Egg	3.70 ± 0.61	142 ± 8
First Instar	5.90 ± 0.62	171 ± 10
Second Instar	4.22 ± 0.76	271 ± 19
Third Instar	6.25 ± 0.89	138 ± 19
Fourth Instar	3.02 ± 0.95	253 ± 40
Pre-pupae	3.84 ± 0.98	125 ± 8
Pupae	5.34 ± 0.92	165 ± 41
Total development time		1266

*SE of T_o and DD determined from Campbell *et al.* (1974).

The average minimum temperature threshold for development was 4.6 °C with values ranging from 3.02 °C for the fourth instar stage to 6.25 °C for the third instar stage. A total of 1266 DD was required for development of eggs to adults. Developmental estimates for the second and fourth instars were 271 DD and 253 DD, considerably longer than the estimates for the other stages (Table 4.5). The longer fourth instar stage may be related to the insects' requirement to feed for a longer period to accumulate reserves, to enhance survival during pupation.

4.3.1.2 Adult longevity

Adult longevity decreased significantly as temperature increased ($F_{4, 70} = 8.367$, $p < 0.001$). Longevity ranged from an average of 105.4 ± 25.09 (mean ± SE) days at 8 °C to 5.2 ± 0.88 days at 25 °C, although considerable variation in survival times within temperature treatments was present (Table 4.6). The variation in survival time was minimal in the warmest temperature of 25 °C, where many insects were deformed and died shortly after emergence from pupation.

Table 4.6 - Mean ± SE, median and range of longevity of *A. orphana* adults (both sexes) reared from fourth instar larvae at different temperatures.

Temperature (°C)	8	14	17	22	25
Mean survival (days) ± SE	105 ± 25.1	74 ± 9.3	48 ± 9.9	27 ± 9.4	5 ± 0.9
Number (n)	10	17	18	18	9
Median survival (days)	53	76	28.5	6	3
Range (days)	42 – 224	13 - 134	8-100	6-117	3 - 8
% Deformed	N/R*	N/R*	N/R*	10	80

*N/R = not recorded

4.3.2 Developmental studies in field conditions

Changes in the lifecycle of *A. orphana* throughout the sampling period at the three different field sites and average monthly temperatures during that time are shown in Figure 4.5– Figure 4.7.

Eggs were present in the field at the commencement of the study in April 1996, and these developed as the weather became cooler. Larvae developed through the four instar stages during winter when average daily temperatures were approximately 10 °C or lower. Two peaks of adults occurred in the field during the first season. The first occurred during late spring and early summer, between October and December. The second peak occurred in the autumn, during March and April. In the second season the first peak was not observed due to missing samples, however, the second peak of adult activity was observed at Arve and Ridgley (Figure 4.5, Figure 4.7).

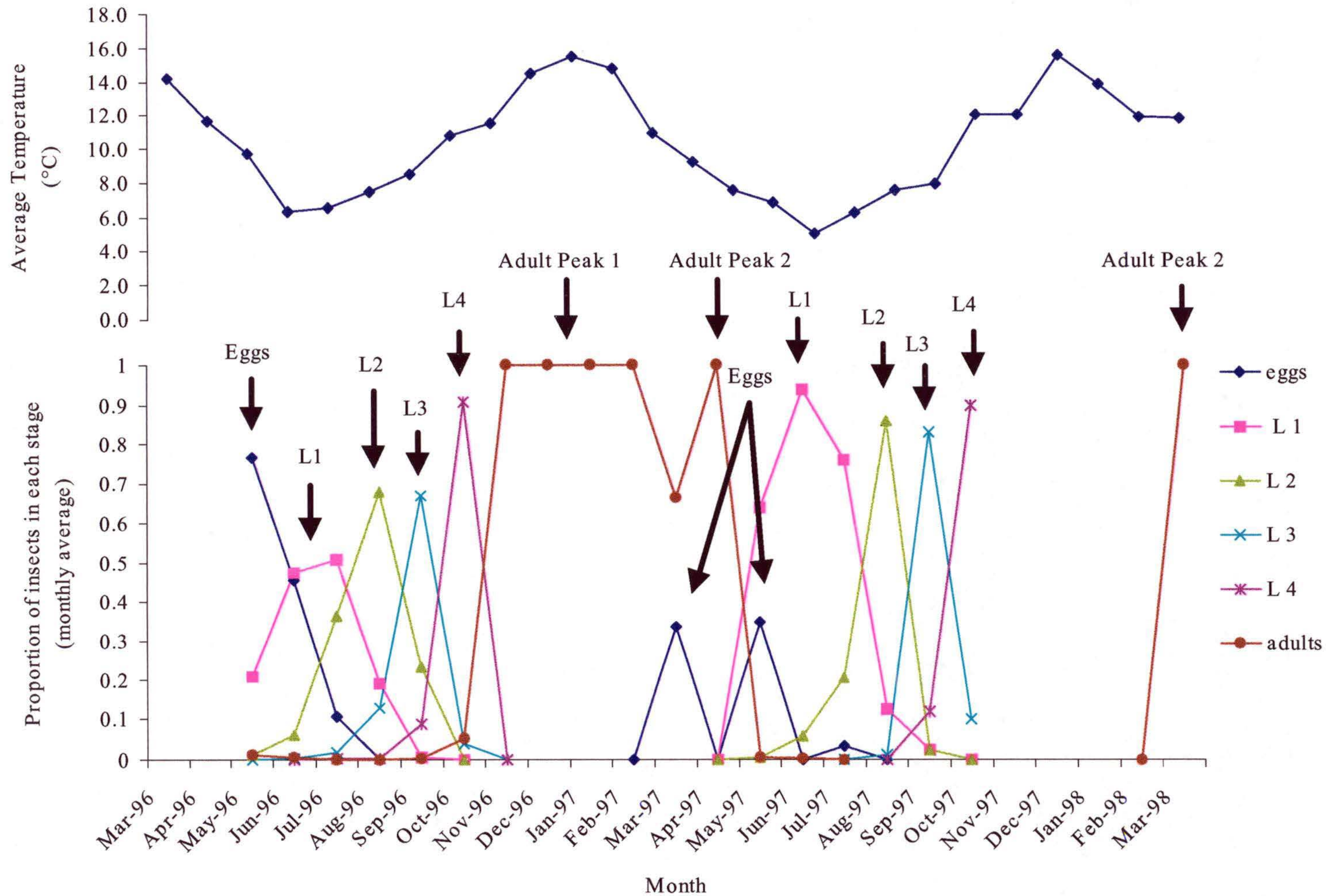


Figure 4.5 – Average monthly temperature and phenology of *A. orphana* at the Arve site between May 1996 and March 1998 Adult peak 1 consists of 74 beetles, and adult peak 2 consists of a total of 91 beetles in the 1996/97 season.

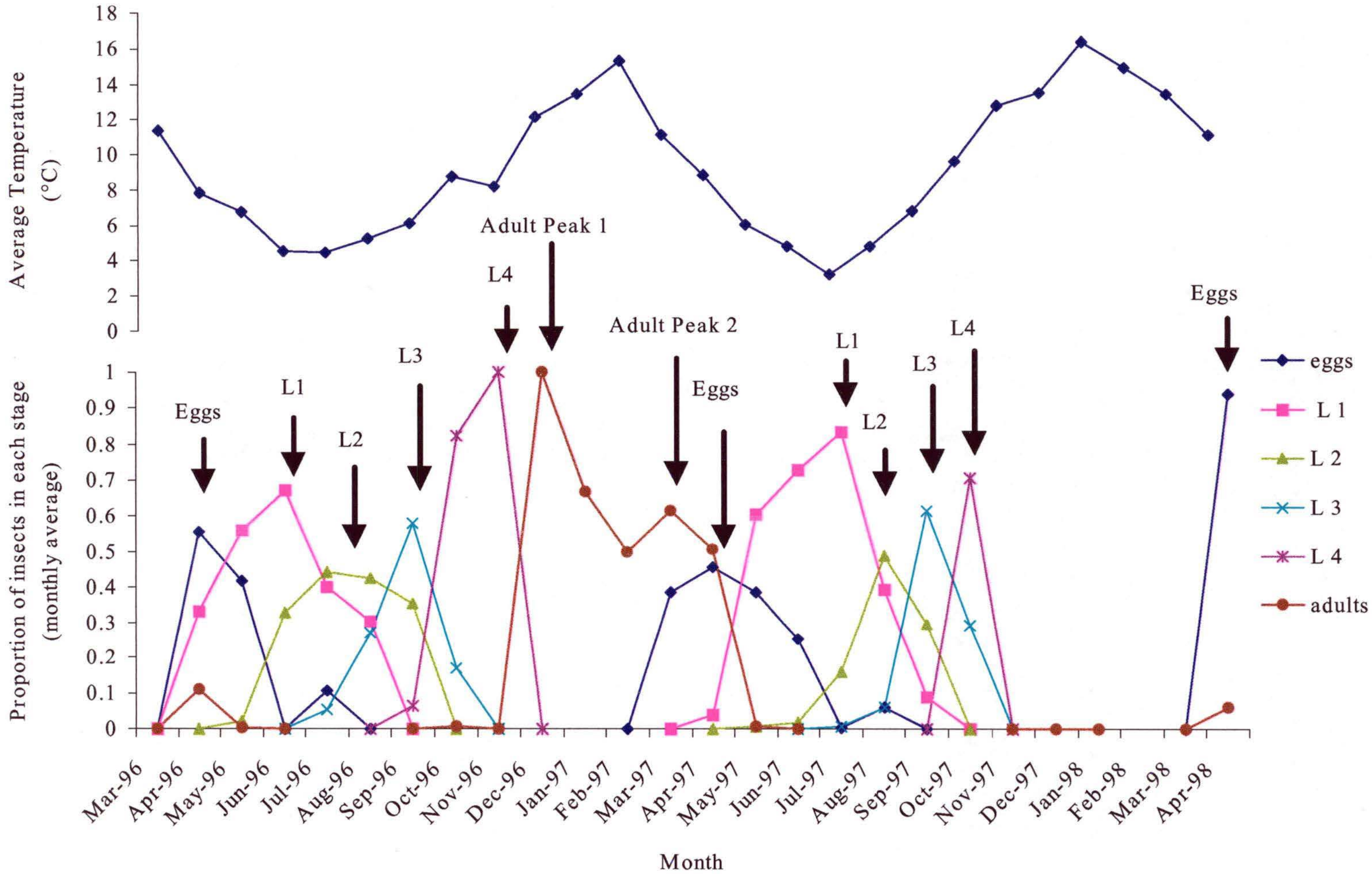


Figure 4.6– Average monthly temperature and phenology of *A. orphana* at the Lake Leake site between March 1996 and April 1998. Adult peak 1 consists of 17 beetles and peak 2 consists of 29 beetles in the 1996/97 season.

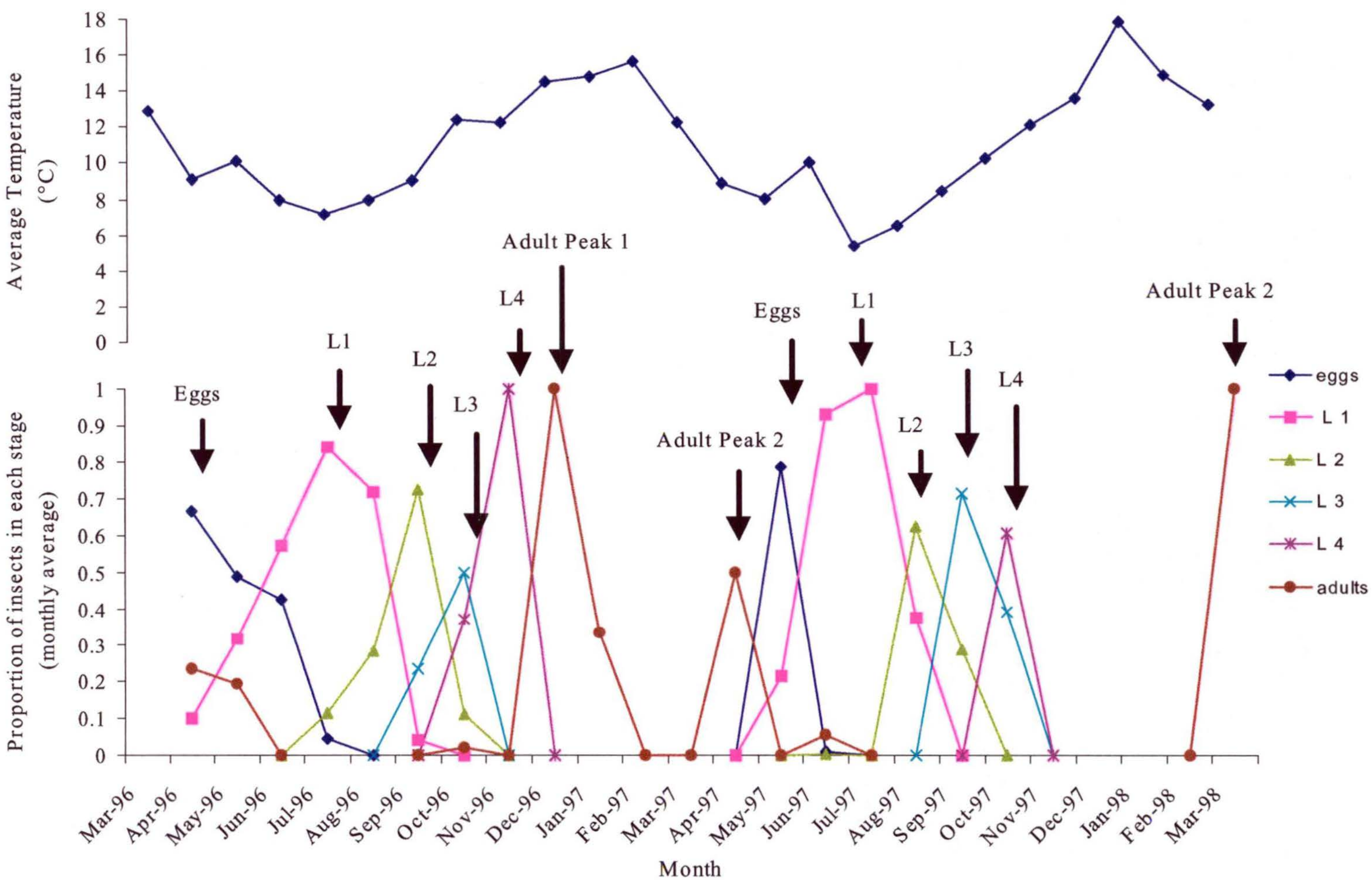


Figure 4.7– Average monthly temperature and phenology of *A. orphana* at the Ridgley site between April 1996 and March 1998. Adult peak 1 consists of consists of 5 beetles and peak 2 consists of 8 beetles in the 1996/97 season

Development times calculated for each stage from the field data are shown in Table 4.7. The linear regression method utilised six data points where available, but during the second season the frequency of sampling was insufficient at the time of the fourth instar and first adult period to accurately model the peaks of these stages. This may have been due to the increased temperatures speeding up development at the time this stage was present in the field, even though the laboratory development data suggests this stage is longer in duration than the other larval stages. Similarly, sampling for the egg stage was only accurate over one season and thus was not modelled using this method. The large standard errors obtained using this method could be improved upon if a greater number of seasons were sampled or, alternatively, if more sites were assessed. The considerable variation in the regressions resulted in minimum temperature thresholds being calculated for the first, second and third instars only. The thresholds were 1.46 ± 0.97, 1.63 ± 0.94 and 4.28 ± 0.85 °C respectively.

In the second method of modelling field development, the value of 4.38 °C was used, as this was the average developmental threshold for all the stages combined in the laboratory (Figure 4.4). Day-degree estimates were calculated by summing temperatures above this value. Also, for comparison, whilst not significantly different ($F_{1,5} = 0.66$, $p = 0.451$), the average day-degree estimates obtained using the variable temperature threshold (determined from the laboratory constant temperature studies) for each insect stage are also included in Table 4.7. Analysis of variance also showed the different stages were significantly different in their development times ($F_{5,17} = 157.24$, $p = <0.001$).

Laboratory results were similar to field results obtained by summation (above $T_o = 4.38$ °C), except in the first and second instar stages where the laboratory results were approximately double the field estimates.

Development times for the two seasons were not significantly different ($F_{1,22} = 1.20$, $p = 0.288$) and so estimates were grouped for the test for differences between sites (Table 4.8). Using this method, developmental times to the first peak of the adults were significantly different, with the Arve site having the longest development and Lake Leake the shortest ($F_{2,17} = 32.50$, $p < 0.001$).

Table 4.7- Developmental estimates for *A. orphana* life stages calculated by a) summation of field temperatures above a variable T_o as determined from the laboratory study for each instar, b) summation of temperatures above an average T_o (= 4.38°C) c) simple linear regression based on field data and d) simple linear regression using laboratory data. All field results are averaged, based on development and temperature data from three sites in Tasmania observed for two seasons.

Stage	Field Summation method Variable T_o (DD)[5]	Field Summation method T_o = 4.38 °C (DD)[4]	Field Linear regression method (DD) ± SE*	Laboratory Linear regression method Variable T_o (DD) ± SE*
Egg	170.3[c]	148.9[c]	N/R[1]	142 ± 8
First Instar	52.9[e]	91.83[d]	252.9 ± 124.7	171 ± 10
Second Instar	122.7[d]	123.9[cd]	254.0 ± 134.9	271 ± 19
Third Instar	106.4[d]	138.6[cd]	108.6 ± 30.5	138 ± 19
Fourth Instar[3]	659.9[b]	553.5[b]	N/R[2]	543
Total time to Adult	1159.5[a]	1104.1[a]	N/R[2]	1266

*SE of DD determined from Campbell *et al.* (1974).
[1]N/R = no result due to too few points in linear regression.
[2]N/R = standard error of estimate was larger than estimate.
[3] average of only one season in the field, in all columns includes pre-pupal and pupal development time.
[4] numbers with the same letter in each column are not significantly different (based on square-root transformed data in Genstat 5 v. 3.2; LSD = 2.266).
[5] numbers with the same letter in each column are not significantly different (based on square-root transformed data in Genstat 5 v. 3.2; LSD = 1.498).

Table 4.8 - Developmental estimates (DD) for each stage in the three different field locations based on summation of temperatures above a minium temperature threshold of 4.38 °C

Stage	Location		
	Arve (DD)	Lake Leake (DD)	Ridgley (DD)
Egg	236.1	61.8	148.8
First Instar	104.6	38.3	132.5
Second Instar	145.6	125.3	96.9
Third Instar	158.4	144.9	112.4
Fourth Instar[1]	746.5	354.6	559.4
Total to Adult Peak 1[2]	1818.2	650.2	1143.9
Total to Adult Peak 2[2]	2117.5	1467.0	1897.9

[1] Includes fourth, pre-pupal and pupal stages.
[2] Based on only 1 season of data.

4.3.2.1 Validating the laboratory model with field data

Actual and predicted dates for the peak of each stage were different at the three different sites, Ridgley (Figure 4.8), Lake Leake (Figure 4.9) and Arve (Figure 4.10). In particular, the laboratory data often predicted stages later than observed in the field for the Lake Leake site, with estimates ranging from 51 to 151 days after the observed peak for the different stages (Figure 4.9). At Ridgley the predictions for the first season were within the range of observed values, but during the second season the predictions varied by up to 90 days later than observed values (Figure 4.8). Similar to the Ridgley site, the results for both seasons at the Arve site were also variable. At this site, many stage predictions were earlier than observed, whilst some stages were later (Figure 4.10). Across locations, the difference between observed and predicted values was greatest in the first, second and third instars. These stages were developing when the average daily temperatures are often close to the minimum threshold temperature. In these conditions, the field located larvae are unable to accumulate day-degrees as quickly as those in the laboratory that do not encounter fluctuating temperatures which are close to or below the threshold temperature. These results suggest that *A. orphana* larvae are capable of increasing body temperature above the ambient temperature during particularly cold times.

Further examination of the Lake Leake data for season 1 suggests that the insects would develop in the observed time using the laboratory development estimates if they were exposed to a T_o of approximately 1.5 °C. As the insects were exposed to an average T_o of 4.38 °C, this suggests that they are able to increase their body temperature by approximately 3 °C through behavioural adaptations, such as basking. The T_o of 1.5 °C is however, similar to that recorded from the linear model of the field data.

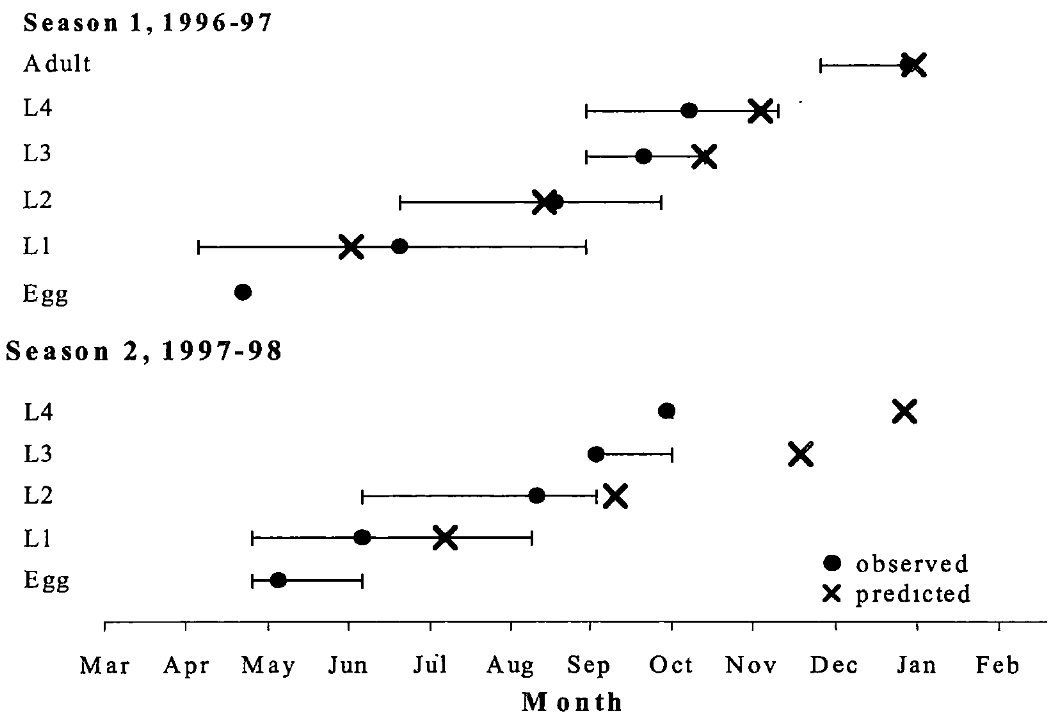


Figure 4.8 - Observed peak and range of each stage and the predicted peak for each stage at the Ridgley site during 1996-1998.

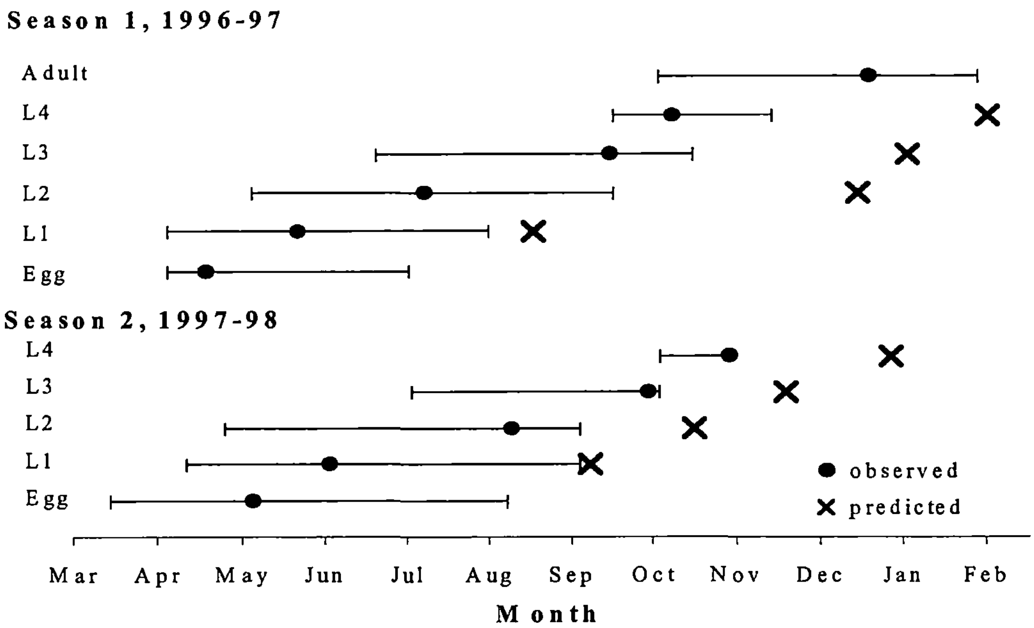


Figure 4.9 - Observed peak and range of each stage and the predicted peak for each stage at the Lake Leake site during 1996-1998.

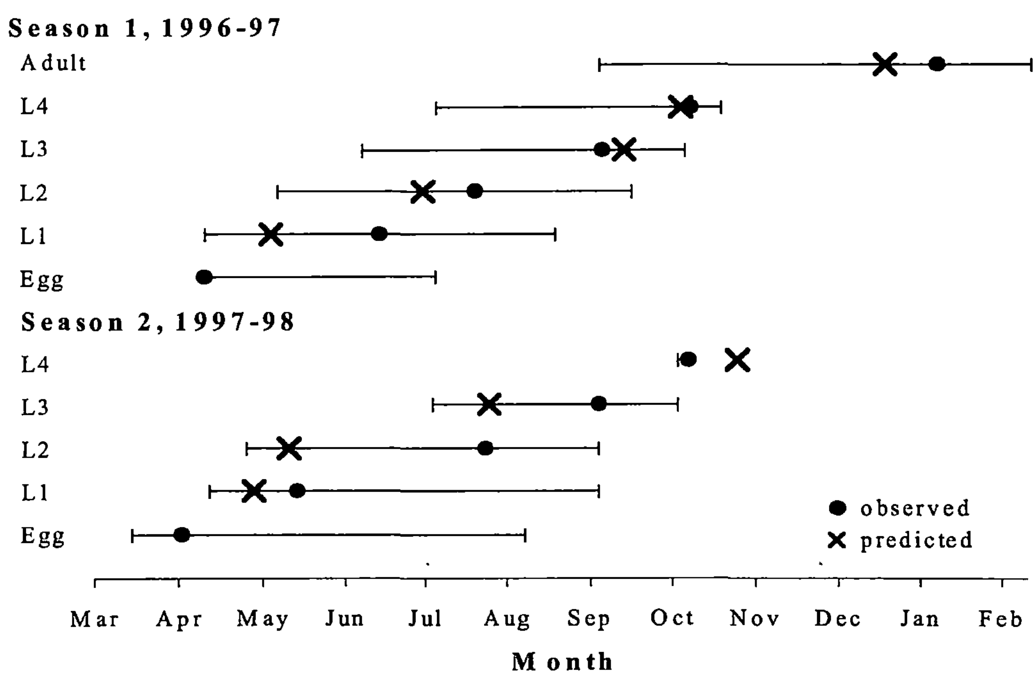


Figure 4.10 - Observed peak and range of each stage and the predicted peak for each stage at the Arve site during 1996-1998.

4.3.3 Reproduction

A total of 2702 (1287♂, 1415♀) adults were collected from the Buckland site during October 22, 1997 and June 9, 1998. Two distinct peaks of adults were observed during this period (Figure 4.11).

Dissection of the insects collected showed that initially similar numbers of males and females were present in the field, but at the start of the second peak the ratio changed to a maximum of approximately 9 males for every female (Figure 4.11). When beetle sizes were assessed to evaluate if females were mating with the larger males (to potentially gain offspring benefit), there were no significant differences observed in male length ($t = 1.17$, $df = 35$, $p = 0.25$), elytral width ($t = 1.18$, $df = 35$, $p = 0.25$) or length x width measurements ($t = 1.36$, $df = 35$, $p = 0.18$) between paired mating and single males near to mating pairs. Also, whilst the sample size is small, only once was the female mating with the largest male on the tree, and on only four occasions was she mating with a male larger in size than the average for the tree. There was no indication of size assortative mating, with the correlation between male and female size in mating pairs not significant ($r^2 = -0.0106$, $p = 0.97$). This preliminary finding suggests that males may not compete for females on the basis of size, and that females do not select for larger males. The small sample size and timing of these measurements at the end of the season may have biased the results. Repetition at the main peak of mating during the season would be worthwhile.

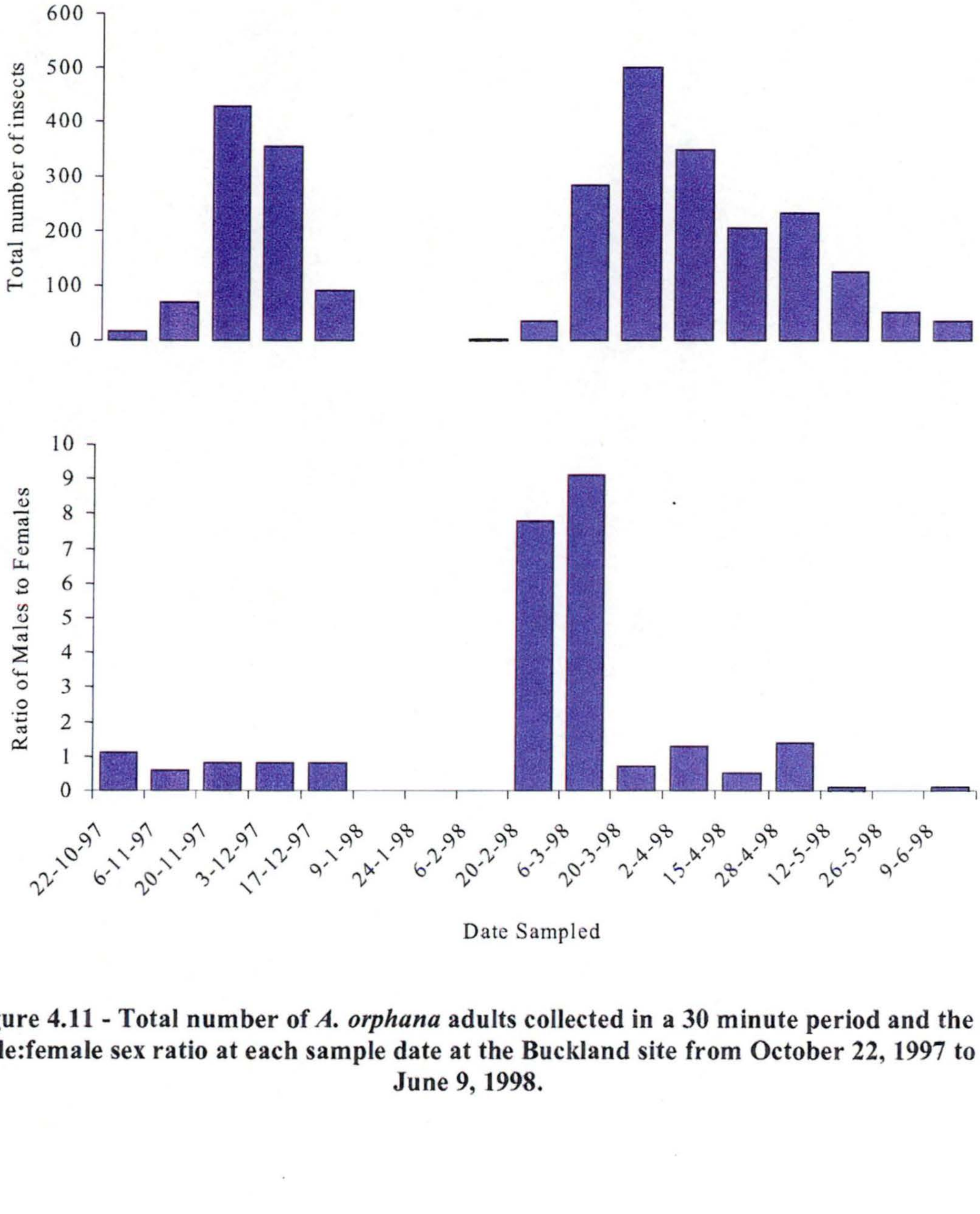


Figure 4.11 - Total number of *A. orphana* adults collected in a 30 minute period and the male:female sex ratio at each sample date at the Buckland site from October 22, 1997 to June 9, 1998.

Table 4.9 -Sizes of mating females, mating and unmated males on trees (Length x Width, mm^2).

Tree	Female size	Mating male size	Un-mated male (n)	Un-mated male average size $\pm$ SE
1	15.9 $\pm$ 0.90	15.0 $\pm$ 0.14	5	14.2 $\pm$ 0.27
2	20.8	12.3	1	14.0
3	16.5	14.3	2	13.9 $\pm$ 0.71
4	20.8	14.8	5	16.1 $\pm$ 0.30
8	16.3 $\pm$ 0.38	11.71 $\pm$ 1.19	7	13.1 $\pm$ 0.16
11	15.3	12.7	4	14.4 $\pm$ 0.28
12	17.6	15.1	3	15.6 $\pm$ 0.41
Total	18.1 $\pm$ 0.61	13.4 $\pm$ 0.58	27	14.0 $\pm$ 0.25

No development of the ovaries occurred during the first peak of adults, but most females exhibited at least some ovarian development during the second peak (Figure 4.12). Ovarian development was apparent approximately one month into the second peak after the male:female sex ratio was at its maximum.

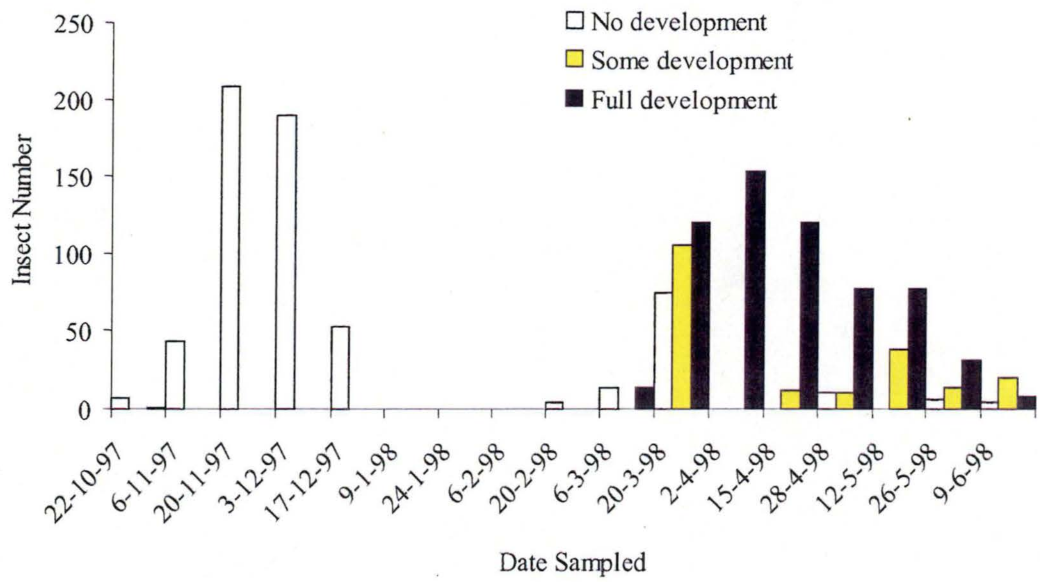


Figure 4.12- Ovarian development of *A. orphana* insects during October 22, 1997 and June 9, 1998.

Insects incubated in the laboratory to examine fecundity experienced high mortality due to contamination with the fungus *Beauveria bassiana.* This reduced the number of insects considerably, and the following results can therefore only be considered indicative.

Females incubated after a single laboratory copulation were able to lay fertile eggs for 46.0 ± 3.75 days. If further mating did not occur during this period, infertile eggs were laid for 35 more days (Table 4.10). Of the 1680 eggs laid, 161 or 9.6 % were infertile. Death occurred at an average of 6.71 ± 0.24 (n = 7, range: 2-11 days) after the last eggs were laid. The total egg laying period of 78 days provides a large window of opportunity for females to mate and lay eggs in the field if they live this long and viable sperm is available. On average, each female laid a total of 140 eggs, with an average egg batch size of 19.5 eggs (Table 4.10).

In comparison, insects that were maintained in cultures of male + female were able to lay fertile eggs for 52 ± 2.69 days (Table 4.10). A total of 148.5 ± 60.17 eggs (n = 8) were laid per female which was slightly more than the single females and the average egg batch size was 20 eggs. No infertile eggs were laid. The relationship between female size and fecundity was not significant (linear regression: $F_{1,11} = 0.58$, $p = 0.460$, $n = 13$), although the sample size was small due to the fungal infection.

Table 4.10 - Duration of egg laying, fertility, egg numbers and batch sizes for female *A. orphana* mated either once or repeatedly.

Treatment	Observation	Average ± SE	Count (n)	Min.	Max.
Females mated once:	- days fertile eggs laid	46.0 ± 3.75	9	30	63
	- days infertile eggs laid	35.8 ± 6.71	5	18	54
	- total days eggs laid	78.2 ± 5.85	5	30	98
	- total eggs laid/female	140.0 ± 29.94	15	9	318
	- egg batch size	19.5 ± 1.48	85	1	55
	- size infertile egg batch	10.1± 0.168	23	1	33
	- size fertile egg batch	22.0 ± 0.054	69	1	55
Male + Female:	- days fertile eggs laid	52 ± 2.69	6	5	63
	- days infertile eggs laid	0			
	- eggs laid	148.5 ± 60.17	8	10	538
	- egg batch size	20.6 ± 4.21	8	1	62

4.4 Discussion

4.4.1 Developmental studies in the field an d laboratory

The main objective of this chapter was to determine the temperature requirements (minimum temperature threshold and day-degree developmental times) for development of *A. orphana* in the laboratory and relate these to field observations. The results would enable managers to predict periods of activity in different climatic areas and thus improve the effectiveness of management programs.

Linear regressions were used to determine the relationship between development rate and temperature for all life stages of *A. orphana* (Figure 4.4), as the development rates were closely related to temperature in a linear fashion between 12 and 22 °C. Development rates at temperatures outside this range were not included in the analyses due to high mortality as shown in Table 4.3. Attempts to rear larvae from eggs at temperatures above 23 °C were unsuccessful as larvae died within a few days of eclosion, possibly due to heat stress. This result was not unexpected, as daily average temperatures rarely exceed 15 °C in Tasmania during the winter when this insect is developing (Figure 4.13). At temperatures lower than 12 °C larvae emerged from eggs, but developed very slowly and died early in the lifecycle. In contrast to the previous observation of weather data, this result was somewhat unexpected, as average daily temperatures are often below 12 °C during the Tasmanian winter (Figure 4.13). This may suggest that the insects have made adaptations to aid their development during the cold winter, such as basking (Maddox, 1995) which is discussed in more detail later in this chapter.

The developmental threshold (T_o) for the different life stages ranged from 3.02 °C for fourth instars to 6.25 °C for the third instars. The overall T_o for eggs - adult emergence was found to be 4.38 °C (Figure 4.4, Table 4.5). The average minimum threshold value obtained is comparable to those obtained for other paropsines, such as 6.3 °C for *P.atomaria* (Carne, 1966a), 11.6 °C for *Ps. tigrina* (Maddox, 1995) and 3.95 °C for *C. bimaculata* (Clarke, 1998). It should be noted however, that these are summer developing paropsines whilst *A. orphana* is a winter developing paropsine and thus it could be expected that its T_o would be lower, unless adaptations to winter development have been formed so that survival of the population is not adversely affected by the low winter temperatures.

The developmental time for the duration of the whole lifecycle was found to be 1266 DD from newly laid eggs to emergence of adults. Considerable variation was observed between the lengths of the different stages, with the shortest stage (pre-pupal) being only 125 DD. The longest stages were the second and fourth instars, at 271 and 253 DD. The third and fourth instars are present on the trees in the spring, and may do considerable damage to the host *A. dealbata* (Elliott, 1978; pers. obs.), and hence control needs to be exercised before the third instar, at approximately 584 DD after oviposition according to the laboratory model. Furthermore, the hosts growth flush is commencing around the time of the peak of the third instars. Although this enhanced host growth may be of nutritional benefit to the later instar stages whilst accumulating reserves for pupation, the overall growth of the host may be reduced due to larval feeding on this new growth. It has been suggested that the evolution of a lifecycle such as this has considerable benefits for the insect, as it utilises the host when it is at its peak for insect nutrition (Mattson and Scriber, 1987; Lawrence *et al.*, 1997).

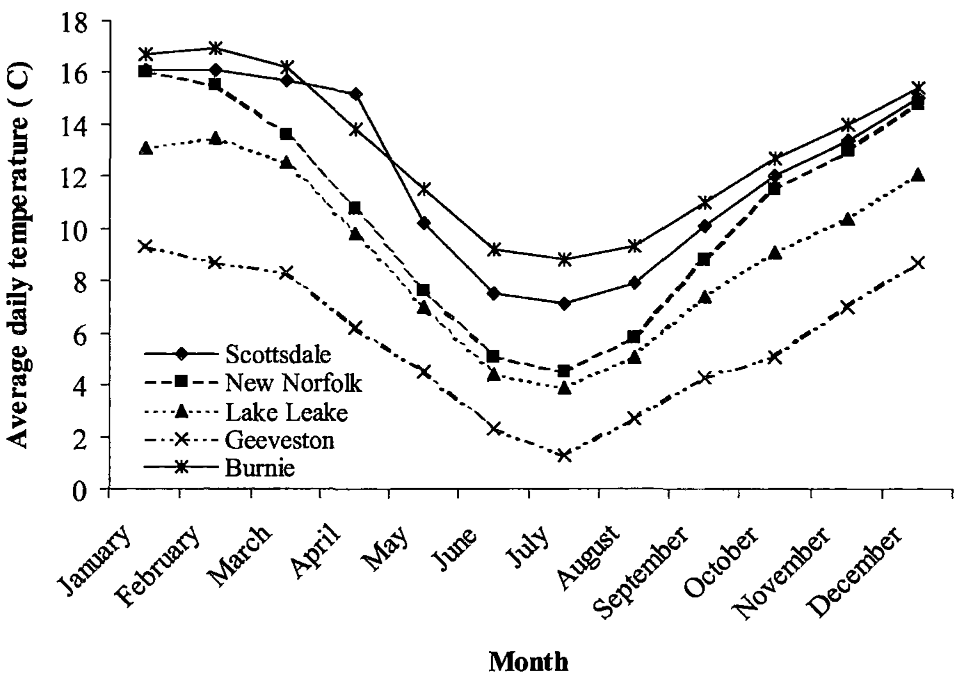


Figure 4.13 – Long term average monthly temperatures at 5 sites where *A. orphana* has established populations in Tasmania. Data from Bureau of Meteorology w.w.w. page, Feb. 2000.

In comparison to the laboratory model, the first field model, which utilised information collected over two seasons from the three sites – Lake Leake, Arve and Ridgley suggested a minimum temperature threshold of 1.46, 1.63 and 4.28 °C for the first three instar stages. Direct comparison of these estimates of T_0 to the laboratory threshold values show that they are lower, whilst the corresponding developmental estimates are higher for the first instar, at 253 DD compared to 171 DD, but comparable for the second and third instars (Table 4.7). Whilst more detailed sampling over a greater number of sites would improve the accuracy of this model, it suggests that in the field, third instars could be expected later than predicted in the laboratory, at 507 DD past the first instar stage.

Due to the lack of information obtained using the first field method, the second and third methods of modelling the field data using summation of temperatures from the predicted peak for each stage were considered more appropriate. These models rely on proportional data that is then modelled to obtain a maximum for each stage which is consistent with the peak of insect numbers for that stage. The idea of modelling development to determine the peak of each stage is similar to that used by Dennis *et al.* (1986) and Kemp *et al.* (1986) but for simplification, a polynomial was used instead of a logistic equation. This method is not as precise as the continuation ratio method used by Candy (1991) as it uses only stage specific data for prediction of a given stage rather than information from all stages.

Once the duration of each stage was known in the field, summing the temperatures above the laboratory estimate of 4.38 °C or the variable T_0 from the individual stages produced similar results, suggesting that use of the simpler constant T_0 of 4.38 °C would be sufficient for future modelling (Table 4.7). These models both suggested the third instars would be present in the field much earlier than the two previous models, at approximately 350 DD. Part of the reduction in time to third instar using these methods is due to the low DD estimates obtained for the first instars. That the calculated development time was low compared to the laboratory estimate may again suggest that the insect is elevating its body temperature in the field by basking, an option that was not available in incubators. Clarke (1998) and Maddox (1995) observed basking behaviour for other paropsines with the latter noting that basking increased the body temperature of *Ps. tigrina* by up to 8 °C above ambient temperatures. Other examples of basking behaviour by insects include the Colorado Potato Beetle (Lactin *et al.,* 1995), the grasshopper *Melanoplus sanguinipes* (Fabricius) (Lactin and Johnson, 1998) and the coreid, *Amorbus obscuricornis* (Westwood) (Steinbauer and Clarke, 1998). The results obtained here suggest that *A. orphana* can raise its body temperature by approximately 3 °C above ambient temperature.

Alternatively, the threshold of 1.5 °C predicted for these early stages by the first field model may suggest that larvae exposed to diurnal or fluctuating temperatures were able to withstand lower temperatures but still develop faster due to the non-linearity of the development model at the top and bottom of the temperature range (Regniere and Bolstad, 1994). As these non-linear sections are overlooked in the laboratory constant temperature model, the need to examine development in the field is highlighted.

Field validation of the laboratory model highlights the need for a longer study on the development of this insect and shows clearly that a model created from laboratory data does not apply to *A. orphana* in the field. Discrepancies between laboratory and field results have also been recorded by Collier and Finch (1996) with their work on carrot fly (*Psila rosae*) and for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Tauber *et al.*, 1988a; Tauber *et al.*, 1988b). In this study, the large variability in the predicted results at the Arve and Ridgley sites may be due in part to the slightly higher daily temperature averages recorded at the locations in comparison to the Lake Leake site which was the coolest of the three (Table 4.2). If the insects are basking and increasing their body temperature when average daily temperatures are close to the threshold, then the estimates based on the laboratory results could be expected to be consistently late, as observed. Further experiments examining the effect of variable daylength, fluctuating temperatures, food quality and basking ability of the insect may help to clarify some of the discrepancies. Soil type, nutrition and growth rate of the trees that the insects were feeding on was not evaluated in this chapter, but these may also affect the insects food source and hence survival and development (*"plant vigour hypothesis"* – Price, 1991). In Chapter 9 the effect of the host on insect growth is investigated further.

Also not apparent from the laboratory study was the presence of two main periods of activity of the adults in the field. The first adult peak was late in November, as the insects emerged from pupation. They then had a period of feeding and flight activity before 'disappearing'. Few adults were observed again, until late in February. Two possible scenarios to explain what may be happening during this period are following. Firstly, the insects emerge from pupation, feed and fly, then enter an aestivation or quiescence period as the weather warms up in the summer. This could be similar to the reproductive diapause occurring during the winter (when it becomes too cold) in summer developing paropsines such as *C. bimaculata* (Greaves, 1966) and *P. atomaria* (Carne, 1966a). But, it is unlikely that *A. orphana* enters diapause, as that is defined as '*a delay in development that is not the direct result of prevailing conditions*' (Torre-Bueno, 1989) and no such delay was observed in the laboratory studies. When the weather cools, they could sexually mature, mate and the lifecycle would continue. Alternatively, a second option may be that the insects emerge from pupation, feed, fly and also mate. The mating may be a cue to commence sexual maturation, which occurs prior to the summer aestivation period. The problem with the second idea is that the study of the sex ratios during the summer period showed males were emerging before females in the second peak, by approximately one month (Figure 4.11). If they had already mated, then there would be little need for this. Also, the female ovaries were not fully developed until approximately one month after the aestivation period, suggesting that it is more likely that mating and egg development occurred after aestivation. Furthermore, male emergence one month prior to female emergence could be to increase the chance that males will find a mate, and so by emerging before the females they are ready and waiting for appropriate mates and any intra-sex competition between males (Shelley and Whittier, 1997) may have already occurred. The preliminary study of mating behaviour conducted here suggested that size has no effect on competition, differing from results obtained by Simmons (1995) in studies of field crickets, *Gryllus campestris* (Linnaeus). However as the study here was done towards the end of the mating season it does not exclude the potential for this behaviour to occur in February, at the commencement of the second peak of adults and when the sex ratio was found to be 9:1 (M:F). Simmons (1995) also suggests that females may select males of better quality to improve the survival and mating opportunities of their offspring, yet in this study the females did not appear to discriminate between large or small males for partners. It may be that *A. orphana* females are able to choose which male they wish to fertilse their eggs if multiple mating occurs using one of several methods outlined in Eberhard (1997). Again, the mating behaviour early in the season when the greatest pressure to mate successfully was not examined, and hence further observations during the peak of mating activity may yield a different result when there is a greater selection of beetles available. Finally, these results contrast with the theoretical ideas of Alexander *et al.* (1997) who hypothesise that a female may choose a mate either directly, based on size or indirectly, based on intra-male competition. However, from the information collected, the idea that females may mate with a male on the basis of his performance in respect to an environmental cue, such as the male being in the 'right place at the right time' does apply to *A. orphana* (Alexander *et al.* 1997).

4.4.2 Adult longevity

Average adult longevity was significantly affected by temperature, with longevity decreasing as temperatures increased. At the highest temperature of 25 °C adults were very short lived (approximately 3 days) and many were deformed. These observations support findings of the development experiments that a constant temperature of 25 °C was stressful for the insects.

The variable longevity of insects within treatments may be attributable to differences in longevity between the sexes, although this was not tested. It is possible that some treatments contained more females and these may have lived longer than the males, as noted by Leddy *et al.* (1995) in their study with whiteflies, *Siphoninus phillyreae* (Haliday). The poor survival of *A. orphana* at the highest temperature treatment provides an indication of the temperate ecological preferences of the insect. The average daily temperature in Tasmania rarely exceeds 18 °C (Figure 4.13) and thus it is highly unlikely that *A. orphana* would be exposed to constant temperatures of 25 °C. Temperatures on mainland Australia are higher than in Tasmania, and thus *A. orphana* may be restricted to cooler areas or higher altitudes. The effect of climate on the geographical distribution of this insect is considered further in Chapter 6.

4.4.3 Reproduction

Fecundity studies showed each female lays approximately 140 eggs which is slightly higher than the 114 ± 8.5 (range 61-190) observed by Elliott (1978) but still considerably less than the numbers of 500-1000 observed for some other paropsines (Carne, 1966a; Greaves, 1966). Average batches consisted of 20 eggs, which is also considerably less than the 50-75 eggs/batch recorded for *P. atomaria* (Carne, 1966a), and may be partly due to the much smaller body size of *A. orphana* (approx. 6mm long) compared to *P. atomaria* (10-15mm long). Carne (1966a) found a relationship between body size and egg batch size in *P. atomaria.* Whilst it was not possible to examine batch size in relation to body size for paropsines in general (due to a lack of information), this relationship could be consistent within the Paropsina. It is also interesting to note that *A. orphana* laid infertile eggs when mating had not occurred after an average of 46 days, although the batch size of infertile eggs was less, at approximately 10 eggs. Dickinson (1997), in an account of Chrysomelid mating behaviour notes that chrysomelids mate several times and store sperm for use as required. The mating behaviour of *A. orphana* appears to be consistent with this, as some females containing fully developed fertile eggs (no data - observation only) were collected three months after the peak of mating and well after most mature insects were absent from the field (Figure 4.12). This may be due to sperm depletion, and thus under field conditions assuming all sperm is viable and mating can be ongoing, there is potentially a 2-3 month period when eggs can be laid if climatic conditions are appropriate. Repetition of the fecundity experiment may produce more robust results, as several females were killed by the fungus *Beauveria bassiana.*

4.4.4 Relationship of the biology and phenology of A. orphana *to geographical distribution and host interaction studies.*

The experiments on development and phenology in this chapter provide basic biological and ecological information on *A. orphana*. The developmental information can be related to the geographical distribution of the insect if climatic parameters are known. Knowledge of the developmental time to the most damaging insect stage enables timely application of management measures and also gives an indication of when to further examine the effect of the insects' intense feeding on its host. This is particularly important with *A. orphana,* as insects are often not observed until trees are severely defoliated and the insects are preparing for pupation. Thus, this chapter provides important ecological background information for the geographical distribution studies in Chapter 6 and the host interaction experiments in Chapters 7 and 8.

5. Natural enemies of *Acacicola orphana*

Abstract

Three natural enemies of *A. orphana* are identified; a fungus, a tachinid and a braconid. The fungus, *Beauveria bassiana* was found in adults in both the field and laboratory whilst the parasitoids were found only in field collected larvae. Preliminary investigations into tachinid parasitism at six geographically different locations in Tasmania showed up to 17% of mortality could be attributed directly to tachinids at any one location and thus, it is unlikely that tachinids would significantly affect the levels of *A. orphana* in natural conditions.

5.1 Introduction

Knowledge of the natural enemies of a pest is an important component in any pest management program. Natural enemies can reduce insect pest numbers such that there is a positive flow-on effect to the host trees. For example, Baker (1998) suggested predatory coccinellids were capable of reducing *C. bimaculata* to below economic threshold levels in *E. regnans* plantations.

As discussed in Chapter 2 (Section 2.7), there are a range of predators and parasitoids having an important influence on the mortality of paropsine beetles (Greaves, 1966; Tanton and Khan, 1978; de Little, 1982; Tanton and Epila, 1984; Mo and Farrow, 1993). Elliott (1978) studied *A. orphana* and recorded only low levels of parasitism, (6 specimens from *"A number of collections... containing several hundred individuals per collection"*) of two tachinid parasitoids, *Deltomyza australiensis* (Malloch) and *Lixophaga* sp. during his study in the Florentine Valley, southern Tasmania. He noted that *A. orphana* larvae develop thoughout the colder months of the year and hypothesised that conditions in the study area were unfavourable for many predators and parasitoids.

The ability of fungal and bacterial pathogens to affect paropsines has also been documented, with de Little (1979a) observing a *Metarhizium* sp. causing mortality in *C. bimaculata*. Elek (1997) successfully used a bioinsecticide, *Bacillus thuringiensis* var. *tenebrionis* (Btt) on *C.*

bimaculata also, and found it killed up to 50% of first instar *C. bimaculata* larvae within four days of application. She suggests that it may be useful for incorporation into a biological control program for *C. bimaculata*. It is likely that fungal and bacterial pathogens exist for *A. orphana*, although no records are present in the literature.

During the developmental studies in Chapter 4 (Section 4.2), three natural enemies of *A. orphana* were observed; a parasitoid, a hyperparasitoid and a fungus. This chapter reports on the identity of these. A preliminary study into the spatial variance of the parasitoid, *Lixophaga* sp. was also undertaken, as this was the most common natural enemy recovered from the beetle and was considered the most likely to have potential as part of an IPM program. The section on the tachinid parasitoid has been published in *The Australian Entomologist* and is included as Appendix 2.

5.2 Materials and Methods

5.2.1 Fungal pathogen

During the ovarian development and fecundity studies in the previous chapter, several adults were collected from the field at the Buckland site (see Chapter 4). Adults collected on December 3 and 17, 1997 were found infected with a fungus upon dissection following death. This fungus was cultured and identified by Dr. R. Milner (CSIRO, Canberra).

5.2.2 Tachinid parasitism

One hundred third and fourth instar larvae were collected by beating *A. dealbata* foliage from a number of trees over a tray from each of six Tasmanian sites between October 9 - 16, 1997. Each site was only sampled once. Prior to this no parasitism of eggs, first or second instar larvae had been observed at any site during the study. Site locations were; Lake Leake (147° 38'S, 41° 58'E), Buckland 1 (147° 44'E, 42° 39'S), Buckland 2 (147° 36'E, 42° 38'S), Conara (147° 27'E, 41° 50'S), Dromedary (147° 06'E, 42° 46'S) and Perth (147° 09'E, 41° 35'S) (Figure

5.1). At all sites *A. dealbata* was the predominant *Acacia* species and no *A. mearnsii* was present. Previously, in the 1996 - 97 season, all sites except Dromedary had been assessed for *A. orphana* defoliation using a visual scoring system, based on 10 trees per site.

Upon return to the laboratory larvae from each site were maintained in one of six ventilated containers under constant conditions (17 °C ± 0.5 °C; 8L:16D; ~66% R.H.). Cultures were cleaned and fresh *A. dealbata* foliage was provided three times weekly. At these times any dead larvae were counted. When cultures were first established, each larva was examined individually and any tachinid eggs on the body were noted. Final counts were made of the number of adult beetles, number of parasitoid puparia and the number of adult parasitoids emerging.

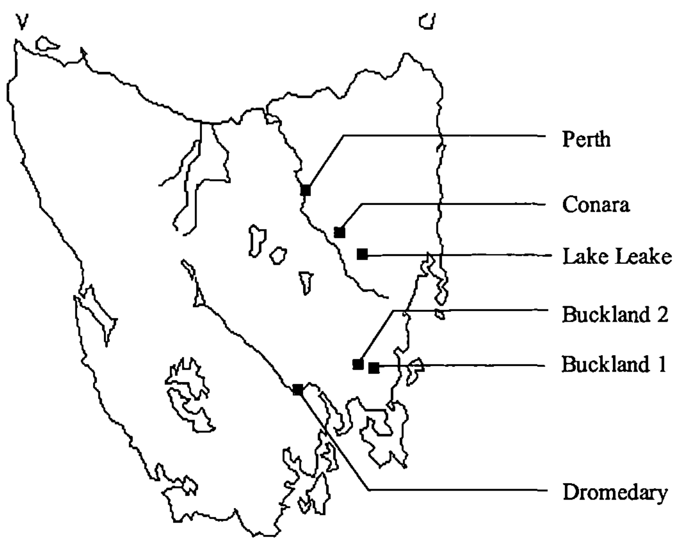


Figure 5.1 - Location of the six sites in Tasmania where larvae were collected for the parasitism study.

5.2.3 Hyperparasitism

In August 1996, a single tachinid puparium was observed protruding from each of two final instar larvae in one replicate of the development study in Chapter 4. The larvae had been incubated at 17 °C and thus the puparia were maintained at that temperature. The puparia were similar in appearance to those of the tachinid *Lixophaga* sp. Two hyperparasitoids emerged from these puparia. Dr. A. D. Austin (Waite Inst., University of Adelaide) provided identifications.

5.3 Results

5.3.1 Fungal pathogen

The fungus was at low levels in the field population, with only 6.7% (n = 12 or 324) of individuals infected on December 3, and 1% (n = 1 of 91) on December 17. In the fecundity trial 11.6% (n = 12 of 105) of individuals were infected. These insects were incubated separately, and thus it is unlikely that contamination between cultures occurred. The fungus was identified as *Beauveria bassiana* (Figure 5.2). Cultures have been included in the CSIRO culture collection held in Canberra, Australia (labels FI – 1273/4/5/6).

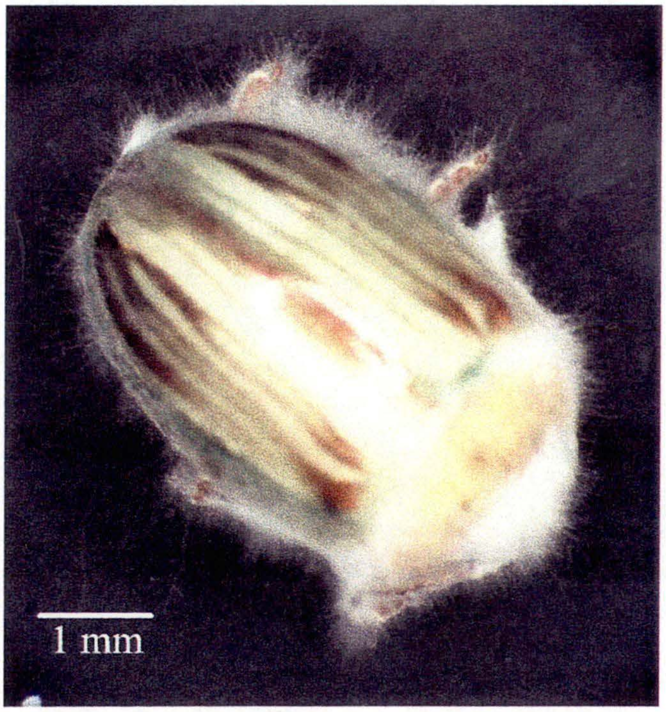


Figure 5.2 - *A. orphana* infected with *Beauveria bassiana*.

5.3.2 Tachinid parasitism

Overall, an average of 5.3% of *A. orphana* larvae collected bore tachinid eggs, with one to three eggs found per parasitised individual (Figure 5.3). Three tachinids developed in larvae collected with no parasitoid eggs on the cuticle, implying oviposition on an earlier instar prior to moult, or that the egg was lost within the stadium prior to collection. The tachinid emerged from and killed the host late in the fourth instar or early in the pre-pupal stage, at which point the puparium could be observed protruding from a split in the hosts' cuticle (Figure 5.4). Adult flies, identified as *Lixophaga* Townsend sp. (Diptera: Tachinidae) (Figure 5.5), emerged after approximately ten days. Only one parasitoid was recorded from each host larva. Prior to death, parasitised *A. orphana* larvae did not appear to behave differently to non-parasitised larvae.

The highest level of parasitism was at Conara (17%) while the lowest was 1% at Perth and Buckland 2 (Table 5.1). Unidentified mortality of beetle larvae in the cultures caused losses of 18-27% (average 24%), higher than the 7.4% obtained for fourth instar larvae in Chapter 4. Thus, some of this mortality may have been caused by parasitoids that failed to emerge. A significant correlation was identified for the relationship between mortality and parasitism recorded (Spearman rank test: df = 5, $r^2 = 0.95$, $p < 0.05$), suggesting that this may be correct, although the sample size was small. From all collections a total of 17 parasitoid pupae were recorded (53 % of those observed carrying eggs), from which only four adult flies emerged (12.5 %). After 15 months the remaining puparia were cut open and well-developed (but dead) flies were observed in all.

A positive correlation existed between the site defoliation scores in the season prior to the collections and the percent parasitism experienced in 1997 (df = 4, $r^2 = 0.98$, $p < 0.001$). Assuming that the level of tree defoliation is related to *A. orphana* levels, this may imply delayed density dependence between beetle and tachinid numbers. Further experimentation and repetition of this experiment with a larger sample size is obviously necessary.

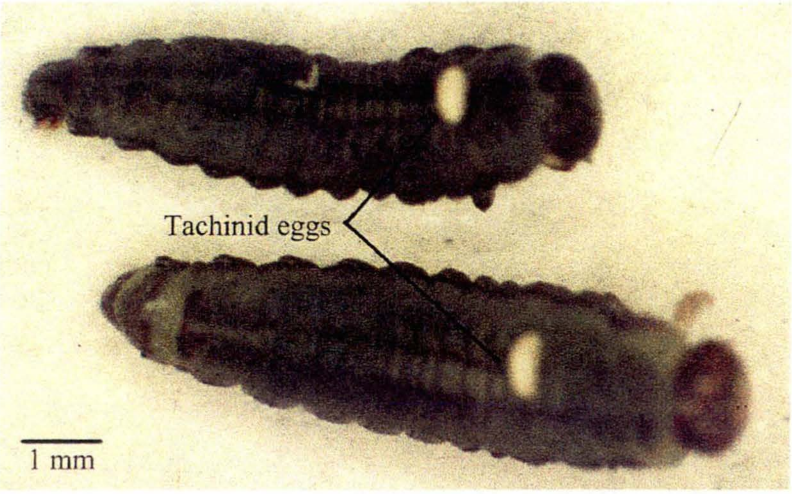


Figure 5.3 - Fourth instar larvae of *A. orphana* with tachinid eggs embedded in the cuticle.

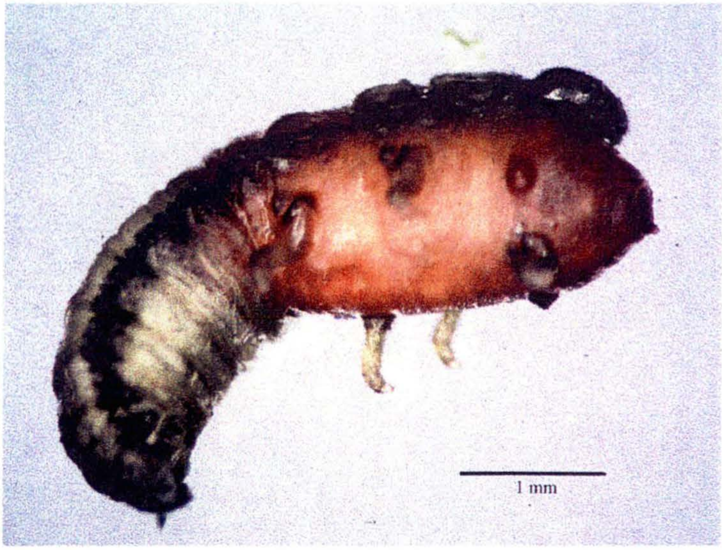


Figure 5.4 - Fourth instar *A. orphana* with tachinid pupa protruding.

Figure 5.5 - *Lixophaga* sp. a tachinid parasitoid of *A. orphana*.

Table 5.1 - Tree defoliation scores and *A. orphana* larval parasitism records for six sites in Tasmania. Initial sample size from each site = 100 larvae, therefore each number can be considered as a percentage.

Defoliation score range; 0 = none - 5 = total defoliation.

Location	Site defoliation score	Larvae carrying tachinid eggs (%)	No. of tachinid pupae emerging	No. of adult flies reared from pupae
Lake Leake	2	2	3	2
Conara	5	17	6	2
Buckland 1	3	8	2	0
Buckland 2	1	1	0	0
Perth	0	0	1	0
Dromedary	-	4	5	0

5.3.3 Hyperparasitism

The hyperparasitoids reared from the tachinid puparia were identified as *Meteorus* sp. (Braconidae : Euphorinae). These have not been recorded previously as hyperparasitoids (A. D. Austin, pers. comm.).

5.4 Discussion

In this chapter, three natural mortality agents of *A. orphana* were identified.

Beauveria bassiana has been used successfully to reduce populations of the chrysomelid *Leptinotarsa decemlineata* in experiments in the USA, causing up to 4-fold increases in potato yield in treated plots compared to those which were untreated (Anderson *et al.*, 1988). Whilst this is an 'environmentally friendly' method of control, issues such as ease of use, and the timing of application need to be considered. There is also potential for large variations in the number of colony-forming units in the formulation (Anderson *et al.*, 1988), and thus dosages are more difficult to measure than in traditional pesticides. The mortality attributed to *B. bassiana* in this chapter shows that it has potential to cause death of *A. orphana*. Further experimentation with this fungus would provide more information on whether or not it could form part of an IPM program for *A. orphana.*

The second mortality agent observed was a tachinid parasitoid of late instar larvae. Larval parasitism (based on the presence of tachinid eggs) averaged only 5% across the six sites sampled, although there was considerable variation between sites (range 1-17%). The results obtained are difficult to compare to those obtained by Elliott (1978) (because of the way his information is presented), but the parasitism rate appears similar. Unlike Elliott however, only one parasitoid species, *Lixophaga* sp., was obtained. Although other authors have recorded low parasitism rates of paropsines at a variety of locations, tachinid parasitism rates of species such as *C. bimaculata* and *P. atomaria* are normally 2-7 fold higher than recorded here (Tanton & Khan, 1978; de Little, 1982; Tanton & Epila, 1984; de Little *et al.*, 1990). The reason for the low parasitism obtained may be due to the winter cycle of *A. orphana* larvae, as suggested by Elliott (1978), but this is difficult to test directly. Alternatively, a greater temporal sampling of larvae may show seasonal cycles or differences in parasitism between years. Seasonality of tachinid parasitism rates has been demonstrated for *P. atomaria* (Tanton & Khan, 1978; Tanton & Epila, 1984).

The low primary parasitism rate, coupled with the low number of mature parasitoids successfully developing and emerging from *A. orphana* larvae, suggests that this species may be a relatively poor host for the *Lixophaga* species observed. Other species in the tribe Blondeliini in Australia have more than one host (Crosskey, 1973) and Arnaud (1978) lists several *Lixophaga* species that use hosts from the Orders Hymenoptera, Lepidoptera and Coleoptera, and one species that utilizes hosts from all three orders. Furthermore, the short (approx. 10 day) pupation time suggests that an alternate host would be necessary to ensure survival of the insect throughout the remainder of the year and thus it is likely that this parasitoid has more than one host.

More studies on field parasitism are required to quantify the effect of this parasitoid on populations of *A. orphana*. *Lixophaga* sp. appears to be an unsuitable control for *A. orphana* as it does not cause death in the host until after the larvae stop feeding, and by that stage they have already caused substantial damage to the host trees. Thus, any benefit to the host trees due to the parasitoid is not obtained until the following season.

There may be several reasons why older instars of *A. orphana* are attacked by *Lixophaga* sp. Later instar *A. orphana* larvae feed voraciously on the host trees and this may result in greater emission of volatiles, which may act as attractants to the parasitoid. Host-finding cues have been identified in other parasitoids for example, *Cotesia marginiventris* (Cresson) which is attracted to *Helicoverpa zea* (Boddie) larvae on corn following the emission of volatiles caused by the larval feeding (Cortesero *et al.*, 1997). Alternatively, the parasitoid may require hosts of a certain size to develop in, and *A. orphana* larvae only reach this size in the later instars. Also, early instar larvae have a higher natural mortality rate than the later instars and thus the parasitoid may select older hosts to reduce the risk of the host dying of other causes before the parasitoid has developed.

The hyperparasitoid, *Meteorus* sp. may also reduce the level of tachinid parasitism although only two individuals were reared from several hundred larvae. However, whilst in this instance it was observed as a hyperparasitoid, it has been known previously only as a primary parasitoid of Coleoptera and Lepidoptera (A. D. Austin, pers. comm.) and may yet prove also to be a primary parasitoid of *A. orphana*.

In conclusion, whilst three natural enemies have been identified, these appear to be unlikely to significantly affect field population levels of *A. orphana* in plantations without supplementation. Further observations determining the temporal distribution of the tachinids are needed to further understand the true impact of this species on *A. orphana*.

6. Geographical distribution of *Acacicola orphana*

Abstract

The distribution of *A. orphana* and its two main host species *Acacia dealbata and A. mearnsii* were mapped throughout Tasmania. A one-off survey of *A. orphana* distribution in southeast mainland Australia is also presented. In Tasmania, beetles were found in most areas where the host species were present. There was no relationship between defoliation and elevation (ASL) or tree size. On the mainland, climate appeared to limit the distribution in the north and west. Using the survey information, the distribution in southeastern Australia was modelled using CLIMEX, a climate modelling package. The potential distribution of *A. orphana* in other continents was predicted using the model created.

6.1 Introduction

Most paropsine species are thought to be endemic to Australia. Some species have become established in other countries including *T. tincticollis* in South Africa (Tribe and Cillie, 1997), *P. charybdis* in New Zealand (Styles, 1970), *T. sloanei* in the USA (T. Paine, UC Riverside, pers. comm.) and *Paropsides* sp. in South-East Asia (Selman, 1994b). The spread of these insects may be linked to the increased distribution of host species in other countries that also have a favourable climate for the insect. *Acacia* plantations have been established in several African and Asian countries including Thailand (Wichiennopparat *et al.*, 1998), Sri Lanka (Midgley and Vinekanandan, 1986), China (Zhigang and Minquan, 1986), Zimbabwe (Gwaze, 1986; Muneri, 1997), Tanzania (Kessy, 1986) and South Africa (Chaunbi, 1997). Stein and Tonietto (1997) also report on plantations in Brazil. In these countries, *Acacia* products are used for tannins, building supplies and firewood. Whilst *A. orphana* has not yet been identified in these countries, it is not known whether conditions are acceptable for establishment should introduction occur. *Acacicola orphana* has been recorded as '*plentiful in Victoria and Tasmania*' (Blackburn, 1898) but no other information regarding the geographical distribution is known within Australia, nor its potential geographical distribution in other countries where host

Acacia species have been established. The studies in this chapter focus on the known geographical distribution of *A. orphana* and the predicted distribution, modelled using climatic parameters. Particular emphasis is placed on the geographical distribution of the insect and *A. dealbata* and *A. mearnsii* in Tasmania.

6.1.1 Climate and geographical distribution

The geographical distribution and phenology of an insect is determined by climate (Andrewartha and Birch, 1954) and is often linked with that of its host species (Southwood, 1973; Nash *et al.*, 1995; Davis *et al.*, 1996). Host distribution is also affected by climatic considerations such as temperature and moisture (Cawood, 1996), which vary with latitudinal differences (Davis *et al.*, 1996). Sutherst and Maywald (1985) developed a computer modelling package (CLIMEX) that has been used to predict the geographic distribution of organisms including the European Wasp, *Vespula germanica* (Fabricius) (Tribe and Richardson, 1994) and the Queensland Fruit Fly, *Bactrocera (Dacus) tryoni* Froggatt (Yanow and Suthurst, 1998) based on climatic data. In CLIMEX, the user can develop a model based on a known geographical distribution and then use this to predict the presence of insect populations in other locations. Alternatively, the user can compare long term climate averages of known favourable locations with unknown locations to determine where a climate similar to the one known to support the insect exists. The similarity between the location climates is indexed with a value between 0 and 100, where 0 is no similarity and 100 is a perfect match.

6.1.2 The Australian climate and environment

Australia is located in the Southern Hemisphere between 10°S and 44°S latitude. Temperatures in Australia are affected by the surrounding oceans, latitude and daylength (Cawood and McDonald, 1996) which, along with the variable and often seasonal rainfall, affects plant species distribution (Davidson, 1934, 1935, 1936).

Australia's topography is low and approximately 70 % of the landscape is within 610 m ASL. Much of the elevated land is associated with the Great Dividing Range, which extends down the east coast through New South Wales, Victoria and into Tasmania. This mountain range is largely responsible for modifying the rainfall in the eastern part of the country (Cawood and McDonald, 1996).

Soil types vary throughout the country, from sandy, free draining soils through to heavy swelling clays and this affects the vegetation. Generally soils are low in nutrients and thus many plants have evolved mechanisms to enhance survival (Cawood, 1996).

Tasmania is a small island located off the southeastern tip of mainland Australia, between 40°S and 43.5°S. It is the only part of Australia to be influenced by the southern maritime air masses and these cause the climate to be mild in comparison to regions of similar latitude in the northern hemisphere (Anon. 1996). The topography is variable and there are strong east-west climatic gradients (McQuillan and Ek, 1996). Rainfall is more uniform than experienced in much of the mainland but decreases moving east-ward across the state, ranging from approximately 2400 mm annually in the west to 500mm annually in the east (Anon., 1996).

Approximately 44% of Tasmania is covered by forests (Anon., 1996), the predominant species are *Eucalyptus* although several *Acacia* species are also common.

6.1.3 Chapter aims and outline

Little information exists on the distribution of *A. orphana* in Australia or on the climatic conditions that may restrict its distribution to southeastern Australia. Before establishing *A. dealbata* plantations, knowledge of its distribution in Tasmania would allow forestry companies to establish plantations in areas with a lesser potential for infestation. Also, with the ongoing development of *Acacia* plantations in other countries, a prediction of the potential global geographic distribution may indicate regions where the insect may become a pest. Hence, for this chapter, the following aims were identified.

1. To determine the distribution of *A. orphana* in southeastern Australia.

2. To predict the potential distribution of *A. orphana* on a continental- and global- scale using a model developed from the Tasmanian distribution.

3. To examine the infestation pattern of beetles between seasons and the relationship between distribution, tree height and elevation above sea level.

6.2 Materials and Methods

6.2.1 Distribution of A. orphana *in Tasmania*

The distribution of *A. orphana* in Tasmania was surveyed during two seasons, from August 1996 to January 1997 (Season 1) and from August 1997 to January 1998 (Season 2). Results of phenology studies (Chapter 4) indicated that these times were appropriate for surveying as the insects were either late instar larvae or adults and thus would be relatively easy to see on the trees. *Acacia dealbata* was the main tree species sampled for the insect, although if *A. mearnsii* was present, it too was sampled.

Survey sites were 5 - 10 km apart and were usually roadside vegetation. *Acacia dealbata* was common in Tasmania, and thus was usually encountered within the 5-10 km distance. Rarely, *A. dealbata* was not encountered but *A. mearnsii* was, and so this was sampled instead and a note made of the species. The sample points follow the road network predominantly and areas inaccessible to conventional vehicles were not sampled. The south-west region of the state was not sampled due to most areas only being accessible by foot.

At each survey site, up to 10 trees (usually a minimum of 3) were checked for presence of *A. orphana* by beating over a tray or inspection of the foliage. If no beetles or larvae were observed after three minutes of searching, the site was recorded as having no *A. orphana* present. If *A. orphana* were observed, the developmental stage and average level of defoliation (scored visually and rounded to one category only) were recorded for the site (see Table 6.1). Tree heights were recorded for all trees sampled during season 1. An estimate of population size as high, medium or low, based on the number of insects encountered was also made. Site elevation above sea level was recorded in the second season.

Table 6.1 - Defoliation level and tree description. Defoliation scores were averaged for 10 trees at each site. If fewer trees were present then the score was averaged on that number.

Defoliation Level	Tree Appearance
1	No visible damage
2	Minimal amounts of defoliation; no chewing of green bark on terminal shoots. Trees appear healthy.
3	Moderate amounts of defoliation; trees with 50% of the pinnules consumed. Some chewing of green bark on the terminal shoots. Trees burnt red in colour in some areas.
4	Considerable defoliation; many of the pinnules absent from the tree. Green bark chewed from terminal shoots. Tree appearance burnt red with few undamaged leaves.
5	Trees totally defoliated. No leaves present, all green bark chewed from foliage and stems on smaller trees (up to 2 m). Trees burnt red with an appearance similar to trees involved in a fire. Trees appear dead.

During the first season map co-ordinates (latitude, longitude) of the survey sites were derived from 1:100 000 maps upon returning to the laboratory. A Silva XL1000 GPS compass (Silva, Sweden) was used in the second season. This provided latitude, longitude and altitude readings.

Latitude and longitude co-ordinates for the insect and its hosts *A. mearnsii* and *A. dealbata* were mapped using Rangemapper™ v2.0 (Tundra Vole Software, Alaska).

6.2.2 Distribution of A. orphana *on mainland Australia*

From November 28, 1997 to December 4, 1997, the geographical distribution of *A. orphana* was assessed throughout New South Wales, Victoria and southeastern South Australia, in conjunction with an annual survey conducted by CSIRO Entomology staff. The predominant tree species surveyed was *A. mearnsii,* although if *A. dealbata* was present it too was examined. Sampling and scoring procedures were as for the Tasmanian survey. Longitudes and latitudes were recorded from a Garmin GPS compass (Garmin International Inc., Australia). Geographical distribution was mapped using Rangemapper™.

To supplement the surveys, institutions with insect collections throughout southeastern Australia were contacted and a request made for label information of any specimens of *A. orphana* in their collections. Replies from the Waite Institute in South Australia and the Australian National Insect Collection in Canberra provided further information on geographical distribution of the insect.

6.2.3 Modeling distribution of A. orphana

The potential distribution of *A. orphana* throughout Australia was modelled using CLIMEX for Windows v1.1 (CSIRO and CRC for Tropical Pest Management, Brisbane). This package predicts potential distribution and relative abundance of an organism using climatic preferences derived from a known distribution (Skarratt *et al.*, 1995).

To use the model it is necessary to estimate or determine a number of unknown parameters relating to temperature and moisture. Together, these parameters form an 'Ecoclimatic index' (EI) which provides an estimate of the overall favourableness of the location for permanent establishment of an insect species. The parameters used to construct the EI include an annual population growth index that describes the potential for growth of the population during the favourable period of the year and four stress indices. These describe the probability of the population surviving through the unfavourable season and are included in the model as heat, cold, wet and dry stress. It is also possible to have interactions between the four stress indices (Skarratt *et al.*, 1995).

The CLIMEX package contains sample distribution maps that can be adjusted to suit any organism. To model *A. orphana,* a map of Tasmania was created. Temperature parameters estimated from chapter 4 were input into the 'temperate' template provided. The moisture and temperature parameters were then adjusted until the EI of locations known to experience severe defoliation were 90 or greater and areas where the insect was not observed had EI's of 50 or lower. A facultative summer diapause was included also, to account for the summer aestivation of adults, which was observed in Chapter 4. Cold, heat, dry and wet stresses were also applied. In the east, the potential for population establishment was further restricted using a hot-dry stress.

CLIMEX parameters were adjusted until high EI's were recorded for areas where the insects were known to have a persistent population and the model map was visually similar to the known distribution. Sensitivity of each of the parameters was examined. A linear regression of the average defoliation level for each region versus EI was also performed.

The model was then applied to southeastern mainland Australia, the Australian continent and globally.

Table 6.2 - Explanation of parameters used to determine geographical distribution of an organism in CLIMEX. (For more detailed information refer to the CLIMEX manual (Skarratt *et al.*, 1995).

Parameter	Explanation
DVO	Developmental zero for the organism. Below this temperature no development occurs.
DVI	The temperature at the lower end of the range for optimal development
DV2	The temperature at the upper end of the range for optimal development
DV3	The upper temperature at which no development occurs.
PDD	Degree-days of thermal accumulation required by the organism to complete a generation
SMO	The soil moisture level below which no development occurs
SM1	The soil moisture level at the lower end of the range for optimal development
SM2	The soil moisture level at the upper end of the range for optimal development
SM3	The upper soil moisture level at which no development occurs.
TTCS	The temperature below which cold stress accumulates
DPDO	The average daylength which induces diapause
DPTO	The daily temperature which induces diapause
DPT1	The daily temperature which terminates diapause
DPD	The minimum days required for diapause development
DPSW	Equal to 0 for winter diapause and 1 for summer diapause
THCS	The rate at which cold stress accumulates
DTCS	The cold-stress degree-day threshold. Included to account for diurnal fluctuations in temperature.
DHCS	The cold-stress degree-day rate (rate of DTCS)
TTHS	The temperature above which heat stress accumulates
THHS	The rate at which heat stress accumulates
DTHS	The heat-stress degree-day threshold. Included to account for diurnal fluctuations in temperature.
DHHS	The heat stress degree-day rate (rate of DTHS)
SMDS	The soil moisture level below which dry stress begins to accumulate
HDS	The rate at which soil moisture dry stress accumulates
SMWS	The soil moisture level above which wet stress begins to accumulate
HWS	The rate at which soil moisture wet stress accumulates

Another method of examining potential distribution using CLIMEX is through the use of the CLIMEX 'match climates' function. This compares the climate of a known location with that of unknown locations. The climate data used for this comparison is; maximum and minimum temperature, total rainfall, relative humidity and rainfall pattern. The resulting match index (MI) indicates how similar the climates are based on these indices. In this chapter, the Scottsdale location was selected for comparison with other locations, as this was known to have a persistent and severely damaging *A. orphana* population. The climate of this site was then compared to SE Australia and the Australian continent.

After examining the fit of the predicted distribution to the surveyed distribution of *A. orphana* across Australia using the two different methods, it was decided to use the model created from the known distribution (method 1) to predict the distribution of the insect throughout the remainder of the world.

6.3 Results

6.3.1 Distribution of the host trees, **A. dealbata** *and* **A. mearnsii** *in Tasmania.*

A total of 671 locations were surveyed during 1996 - 1998. *A. dealbata* was observed at 645 of the sites surveyed and was more common than *A. mearnsii* which was present at only 7% of sites sampled. Tree density was low in the western half of the state, but increased towards the east (Figure 6.1). Only 48 observations were made of *A. mearnsii* in Tasmania, indicating that this species was not as abundant as *A. dealbata* (Figure 6.2). Approximately 20% of observations were of trees planted in farm windbreaks, rather than wild growing trees.

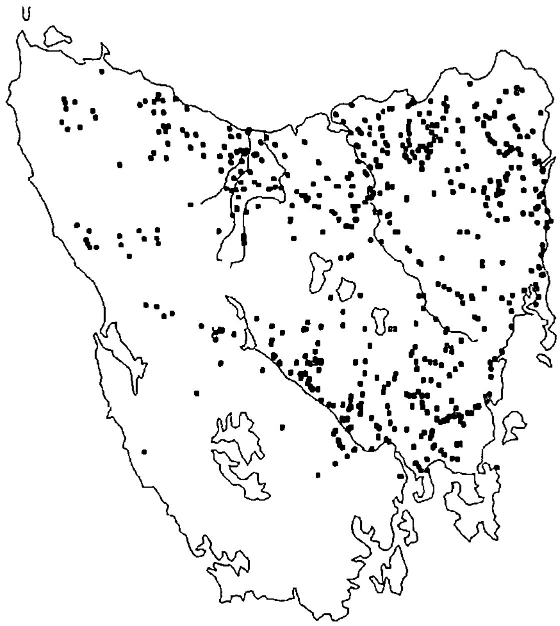


Figure 6.1 - Distribution of *Acacia dealbata* in Tasmania

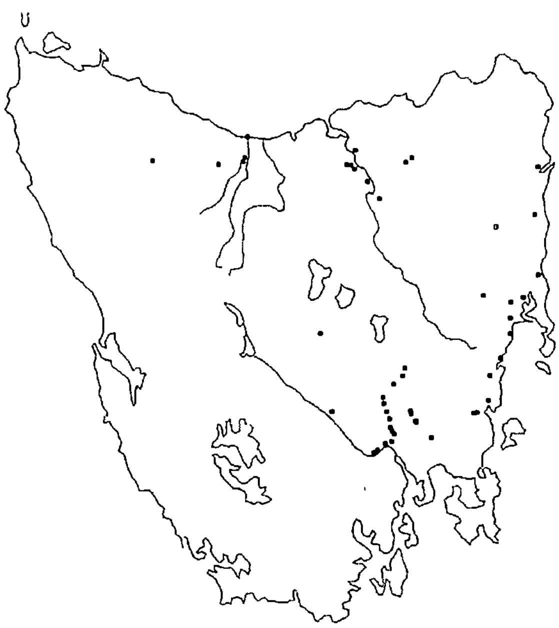


Figure 6.2 - Distribution of *Acacia mearnsii* in Tasmania

6.3.2 Distribution of A. orphana *in Tasmania*

The majority of *A. orphana* observations were from *A. dealbata* trees, with 45% trees sampled infested with *A. orphana* (average for the two seasons). Although there were fewer numbers of *A. mearnsii* in the survey, this species showed only slightly lower infestation levels of approximately 40% (average for two seasons). One record was made of *A. orphana* feeding on *A. baileyana* flowers and foliage, in an area where larvae had already partly defoliated the nearby *A. dealbata* and *A. mearnsii* trees.

During the first season, 412 sites were surveyed for presence or absence of *A. orphana*. The insect was present in 33% of sites examined. Fewer sites (269) were surveyed the following season, and the insect was found in approximately 71% of locations. In the first season insects heavily defoliated trees in the North- and Central-eastern parts of the state (Figure 6.3). Survey results in season 2 indicated that insects were still concentrated in these locations but populations in the south-east had apparently increased as several sites recorded level 5 defoliation scores (Figure 6.4). To keep the figures easy to read, defoliation levels 1 and 2 are not differentiated in Figure 6.3 - Figure 6.5.

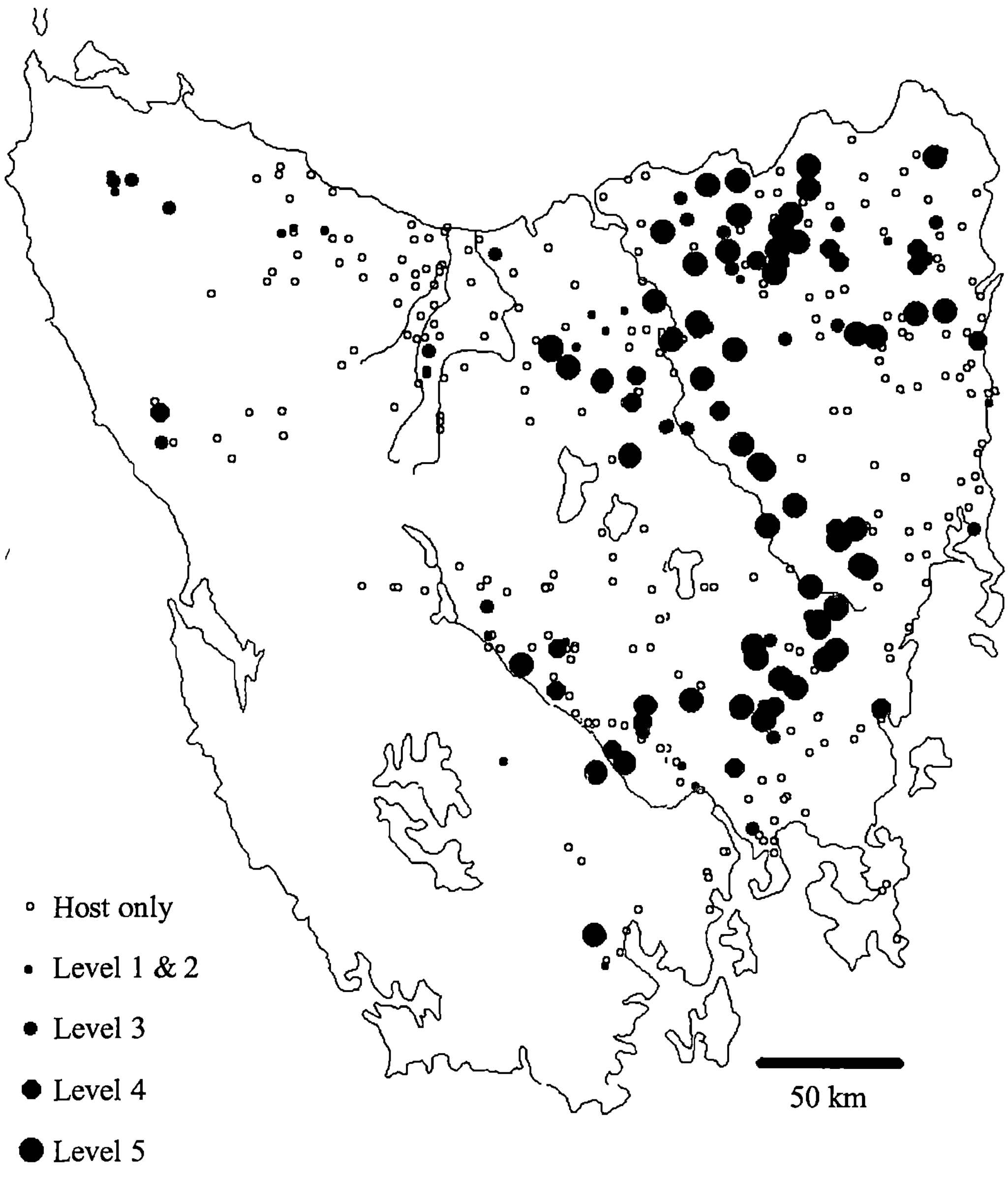


Figure 6.3 - Distribution of *A. orphana* and levels of defoliation in Tasmania during season 1 (1996-1997).

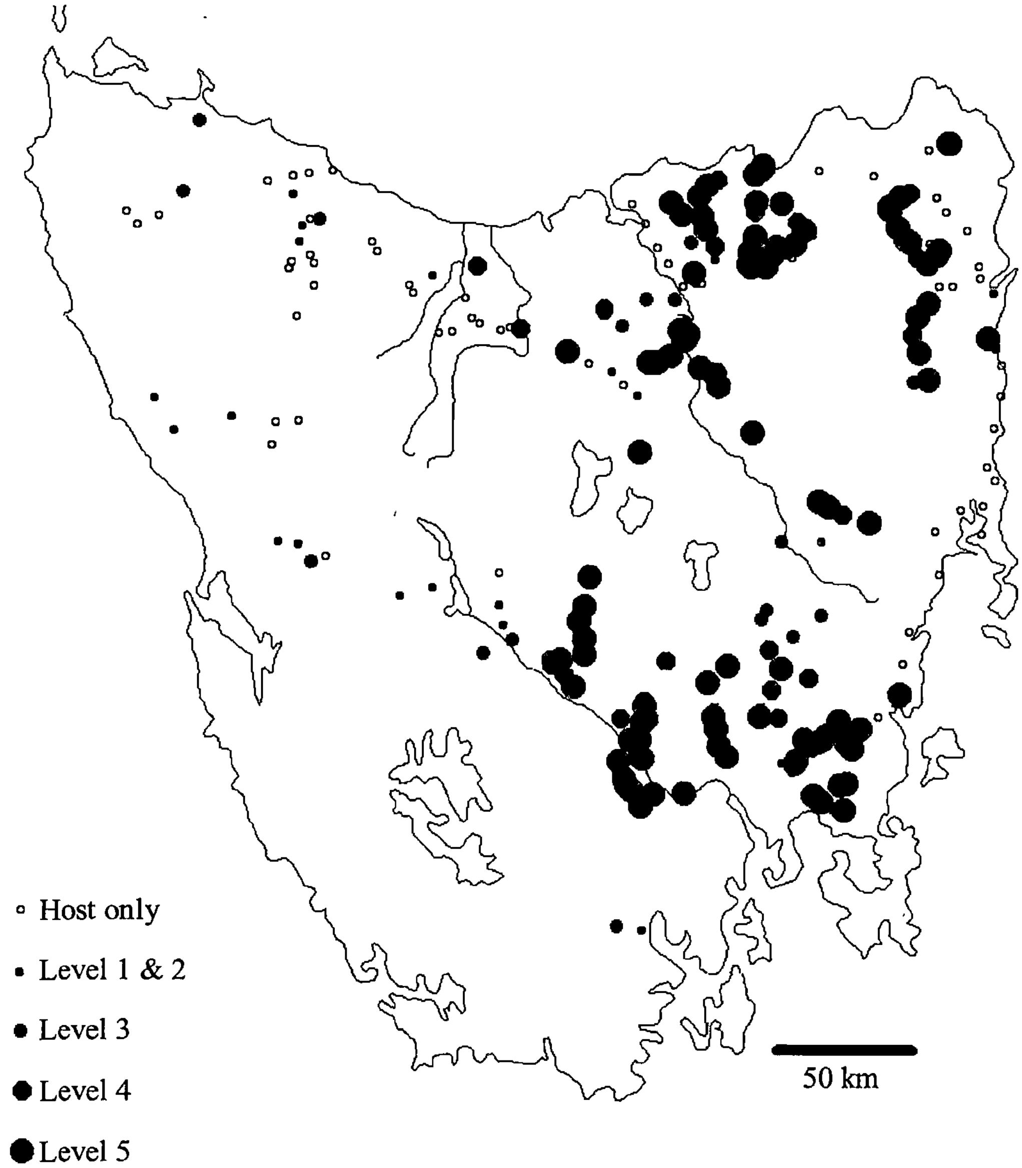


Figure 6.4 - Distribution of *A. orphana* and levels of defoliation in Tasmania during season 2 (1997-1998).

In Tasmania, Table 6.3 shows that more sites experienced level 5 defoliation compared to any other level (as a percentage of total sites surveyed with insects present). No significant differences were observed in the percentage of sites at each defoliation level between the two seasons (t-test; t=2.78, df = 4, p = 0.995). The defoliation levels observed between the two seasons were not significantly different (Contingency table; Pearson $\chi^2 = 3.56$, df = 4, p = 0.469).

Table 6.3 – Percentage of sites at each defoliation level as a proportion of the total infested by *A. orphana* for that season in Tasmania.

Defoliation level range 1 = no visual damage - 5 = total defoliation.

Defoliation level	Season 1: 1996-97 (%) n = 136	Season 2: 1997-98 (%) n = 192
1	7.3	8.3
2	14.0	8.9
3	21.3	15.6
4	16.2	14.5
5	41.2	52.6

A sub-sample of 66 sites throughout Tasmania where exactly the same groups of trees were sampled each season indicated 38% of sites experienced no beetle attack during either season. In the first season *A. orphana* was recorded at 32% of these sites and this increased to 58% in the second season. Coupled with the increase in infestation, the level of defoliation also increased significantly overall, by one defoliation level (ie. the average increased from level 1 to level 2) (t-test; $t = 1.997$, $df = 65$, $p < 0.001$). The relationship between defoliation levels scored in season 1 and the change in defoliation levels between the two seasons was also significant (regression; $F_{1,65} = 9.44$, $p = 0.003$), as was the relationship between damage each season (regression; $F_{1,65} = 19.12$, $p < 0.001$). Few sites were found infested only in the first season (4.5%), suggesting the insects' geographical distribution increased during the second season.

No significant relationship existed between site elevation and defoliation level in Tasmania (regression; $F_{1,191} = 0.455$, $p > 0.05$) or between tree height and defoliation level (regression; $F_{1,266} = 3.78$, $p > 0.05$). The relationship between defoliation level and the estimate of population size as scored at three levels (large, medium and small) was however, significant during the first season (regression; $F_{1,116} = 185.04$, $p < 0.0001$). Population size estimates were not made during the second season.

6.3.3 Distribution of A. orphana *in south-east mainland Australia*

A total of 86 sites were surveyed for presence or absence of *A. orphana* throughout southern NSW, Victoria and southeastern South Australia. Trees sampled were predominantly *A. mearnsii* (96 % of all sites). One observation was made of *A. orphana* adults feeding voraciously on *Acacia decurrens*, in an area where the *A. mearnsii* was already severely defoliated. *A. orphana* was present at 63% of the sites surveyed. Approximately 30% of infested locations experienced level 5 defoliation, the largest of the defoliation classes (Table 6.4). Geographical distribution and defoliation levels are shown in Figure 6.5.

Table 6.4 - Percentage of sites at each defoliation level as a proportion of the total infested by *A. orphana* for the 1997-98 season, recorded during the mainland survey.

Defoliation level range 1 = no visual damage - 5 = total defoliation.

Defoliation level	Sites with that level of defoliation (%) n = 54
1	20.4
2	11.1
3	13.0
4	25.9
5	29.6

When the percentages for proportion of sites infested at the different defoliation levels (Table 6.3 and Table 6.4) were compared across Tasmania and the mainland in 1997-98, the percentages were found to be significantly different from each other (Pearson $\chi^2 = 15.23$, df = 4, p = 0.004).

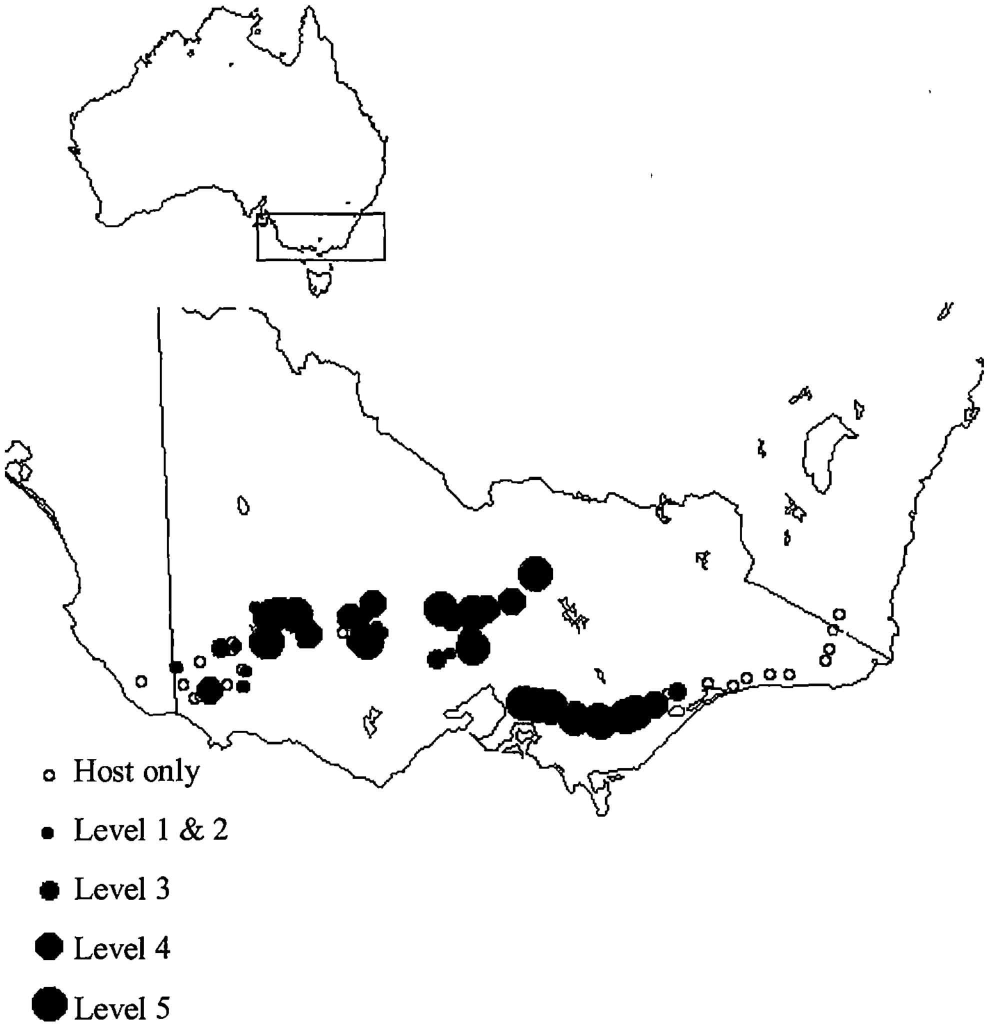


Figure 6.5 - Distribution and defoliation levels of *A. orphana* in southeast mainland Australia.

Data obtained from collection labels expanded the mapped distribution slightly further north along the east coast of NSW, to 10 km south of Monga. It also showed that *A. orphana* was found in the central and central western parts of Tasmania, extending the known distribution in this state (Figure 6.6). No collection specimens were located from South Australia.

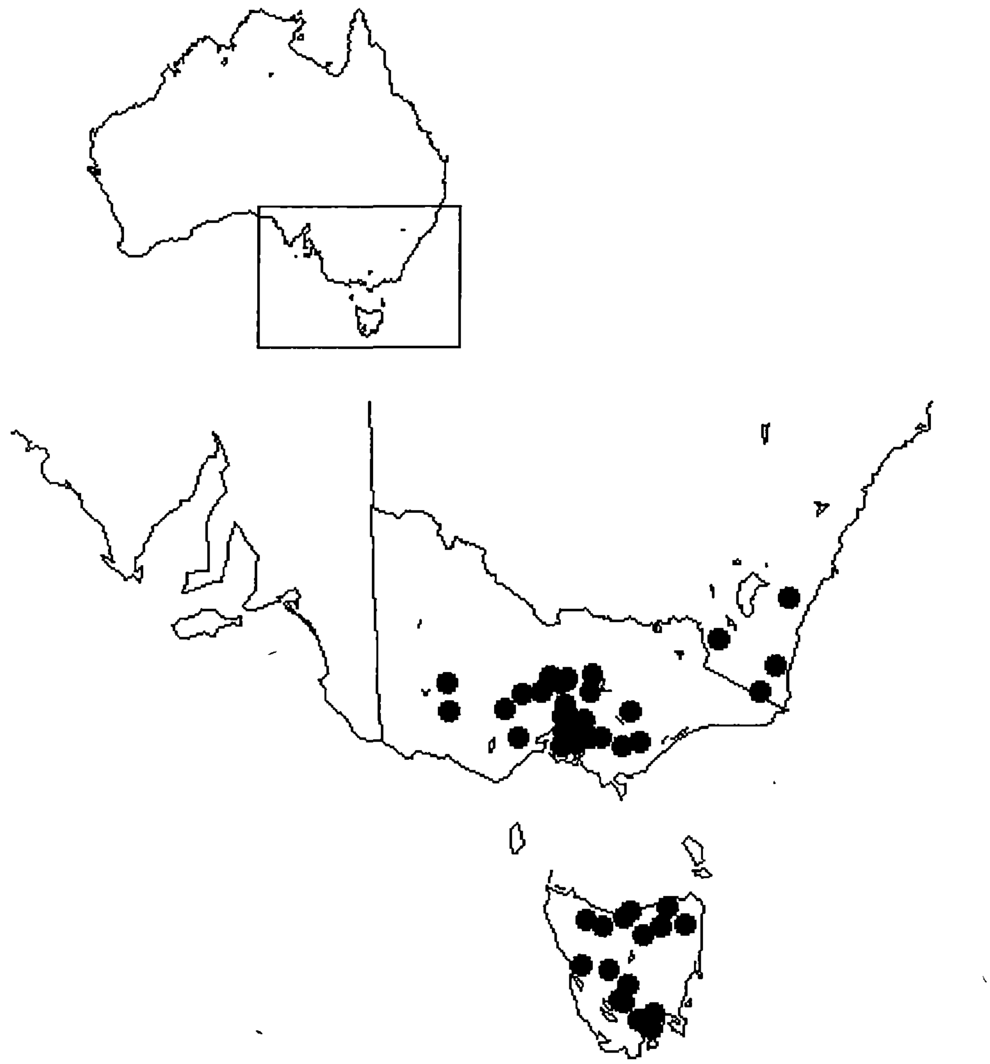


Figure 6.6 - Geographical distribution of *A. orphana* from label data in collections.

6.3.4 Modelling geographic distribution of **A. orphana**

The CLIMEX model and parameters developed using the Tasmanian distribution information are shown in Figure 6.7 and Table 6.5.

Initially, the temperature parameters from Chapter 4 were used for the temperature component of the model. Sensitivity analysis showed that these were not optimal, and hence the minimum threshold (DVO) was reduced from 4.38 °C to a more appropriate value of 1.6 °C. Variation of the parameters DV2 and DV3 had a negligible effect on the EI values, suggesting that high temperatures were unlikely to affect the geographical distribution of the insect in Tasmania.

Excess soil moisture limited the distribution of *A. orphana* in the west of Tasmania. Lack of moisture was found to limit the distribution only slightly in the east.

The parameters chosen for the facultative summer diapause were based on estimates of temperature and daylength at the time of year when the insect enters it aestivation period. The sensitivity analysis of these parameters showed that large changes in temperature and daylength were required for very small changes in EI. Determination of whether the insect is actually in diapause or not would allow improvement in the accuracy of this section of the model.

The addition of slowly accumulating cold stress reduced the EI values for locations in the centre of Tasmania. Increasing the rate of the cold stress further resulted in an EI of zero for all inland data points. The rate of cold stress accumulation had a greater effect on distribution than the cold stress temperature threshold.

The threshold applied for heat stress had little effect on the distribution, but the rate at which heat stress accumulated did. Increasing the rate of heat stress in the model resulted in EI values of zero for the midland region of the state (Figure 6.7). A similar result was obtained with the dry stress parameter. In both cases, the data points on the extreme east coast were relatively unaffected. Application of a hot-dry stress slightly reduced the distribution in the eastern part of the state.

The wet stress index reduced the EI values of locations in the high-rainfall, west coast region.

Further adjustment of the parameters did not restrict the distribution of *A. orphana* along the east coast or far north-west coast. The incidence of the beetle in these areas during the survey was low and could be attributed to a lack of host species in these areas. The high EI obtained for Butlers Gorge in the centre of Tasmania was also difficult to reduce, as application of stresses to limit the distribution in this area also had a substantial affect on the EI values of locations where the insect was known to have permanent large populations. Butlers Gorge was observed to only have moderate defoliation levels when sampled.

Table 6.5 – The temperate template and estimated Tasmanian parameters for the CLIMEX model for *A. orphana* based on the known Tasmanian distribution.

Parameter	Temperate Template	Predictive Model	Parameter	Temperate Template	Predictive Model
Temperature			Dry Stress		
DVO	8	1.6	SMDS	0.2	0.4
DVI	18	4.5	HDS	0.005	0.005
DV2	24	23	Wet Stress		
DV3	28	28	SMWS	2.5	1.2
PDD	600	1266	HWS	0.002	0.0003
Soil Moisture			Diapause		
SMO	0.25	0.2	DPD0	0	14
SM1	0.8	0.25	DPT0	0	21
SM2	1.5	1.3	DPT1	0	18
SM3	2.5	1.5	DPD	0	0
Cold Stress			DPSW	0	1
TTCS	0	3.4	Hot-dry Stress		
THCS	0	0.00005	TTHD	0	20
DTCS	25	0	MTHD	0	1
DHCS	0.0001	0	PHD	0	0.005
Heat Stress					
TTHS	30	23.4			
THHS	0.005	0.01			
DTHS	0	0			
DHHS	0	0			

Using the match climates function in CLIMEX, the comparison of the climate of other locations in Tasmania with Scottsdale showed that several locations in the state were similar, with most locations having an MI of 70 or higher. Butlers Gorge and Waratah recorded some of the lowest MIs (Figure 6.8). In the field survey, *A. orphana* was recorded at low levels near Butlers Gorge, but a negative result was obtained in the survey for Waratah. The similarity of many of the

Tasmanian locations due to the small size of the island may partly explain the difficulty in improving the accuracy of the CLIMEX model.

The CLIMEX model was then applied to the south-east Australia, where a visual comparison of the predicted geographical distribution was only slightly different from that observed from the survey and collection data. The distribution in Victoria was restricted mainly due to moisture and heat stress. The northern-most point that *A. orphana* was predicted to populate was Kirkconnell, NSW, where the EI was 100 (Figure 6.9). In comparison, when the Scottsdale climate data was matched with other locations in southeastern Australia, several locations had climates similar to Scottsdale and the potential distribution extended further north in Victoria (Figure 6.10).

The CLIMEX model developed using the survey data was also applied to the Australian continent and predicted a lesser distribution of *A. orphana* than might be otherwise assumed from the matching of climates (Figure 6.11). The model predicted populations of *A. orphana* would exist only as far north as Kirkconnell in Australia and at only two locations in Western Australia. These locations had EI values of 51 and 70. Moisture and dry stress restricted the potential of the insect to establish in these areas. All South Australian points had an EI of less than 52, suggesting that *A. orphana* would be poorly able to establish in these areas. When the 'match climates' function was applied to the continent of Australia, the map predicted that the insects' distribution could extend through NSW and into southern Queensland (Figure 6.12). Several points in the south-west region of Western Australia also had similar climates to Scottsdale.

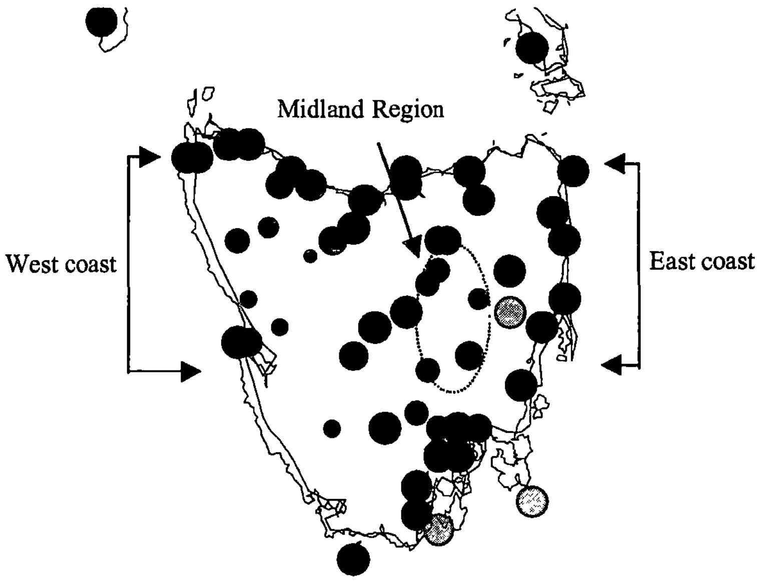


Figure 6.7 – CLIMEX model of the geographical distribution of *A. orphana* in Tasmania. Larger circles indicate a more favourable ecoclimatic index and higher potential for establishment of *A. orphana* populations. Hashed circles indicate an EI of 100.

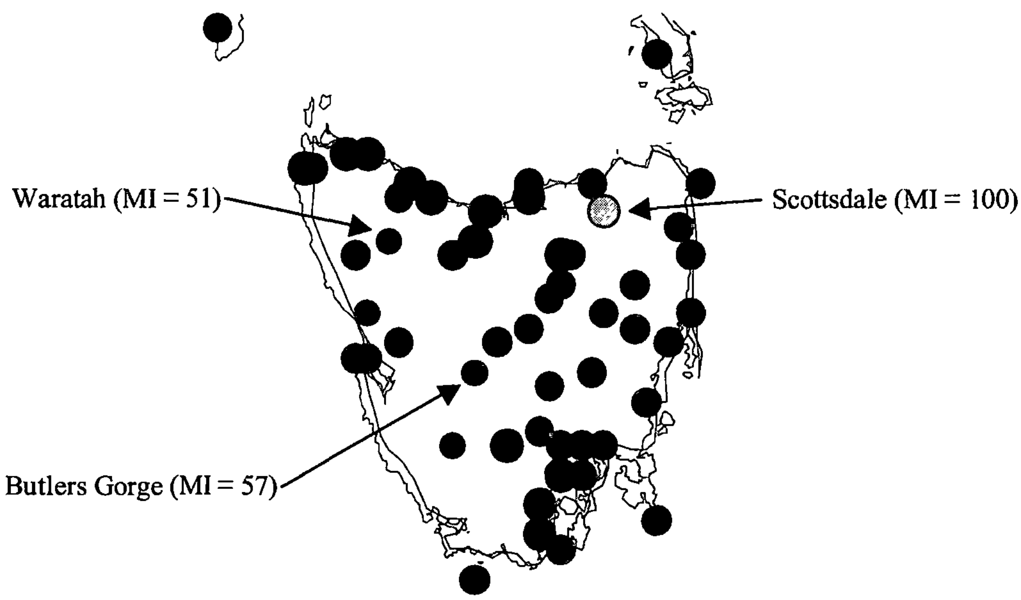


Figure 6.8 – Locations in Tasmania with similar climates to Scottsdale using the 'match climates' function in CLIMEX. Larger circles indicate higher match indices.

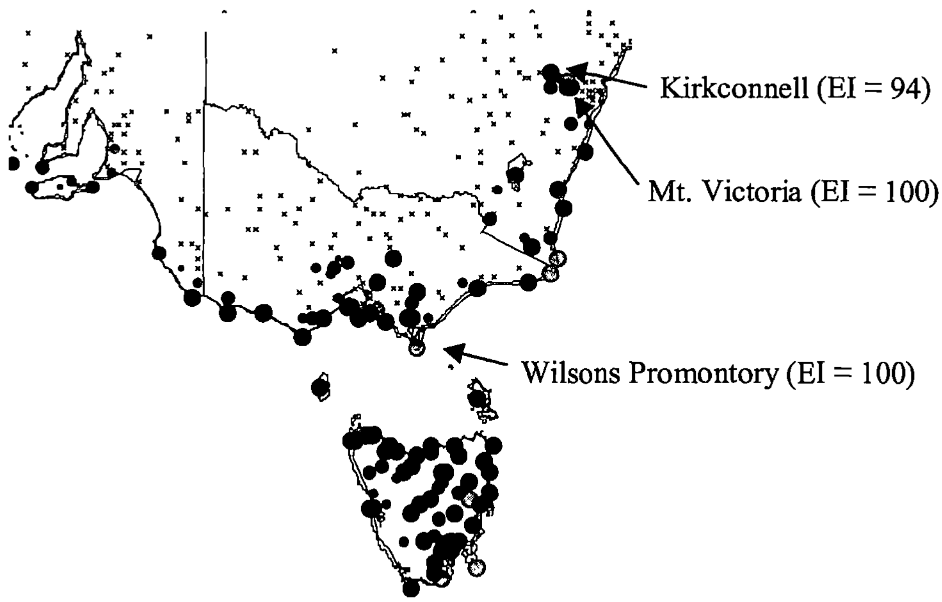


Figure 6.9 - Predicted geographical distribution of *A. orphana* in southeastern Australia using the CLIMEX model created from the Tasmanian distribution. Larger dots indicate higher EI and greater potential for population survival. Small crosses indicate EI = 0.

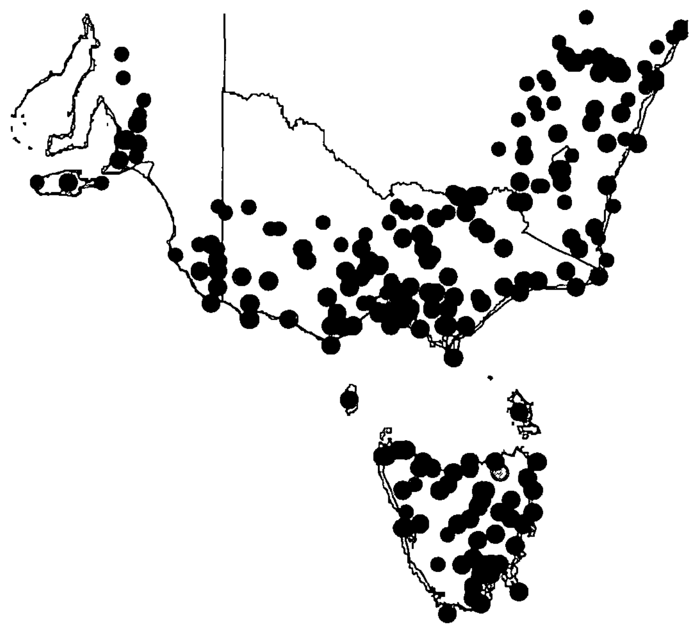


Figure 6.10 - Locations with similar climates to Scottsdale in southeastern Australia using the 'match climate' function in CLIMEX. Larger dots indicate higher MI's.

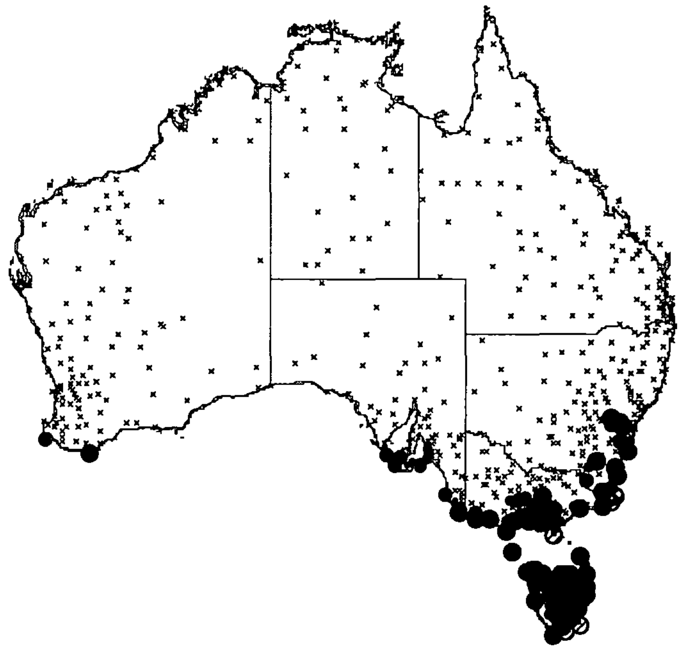


Figure 6.11 - CLIMEX model prediction of the distribution of *A. orphana* throughout Australia. Larger dots indicate higher ecoclimatic index, hashed dots indicate EI = 100. Small crosses indicate EI = 0.

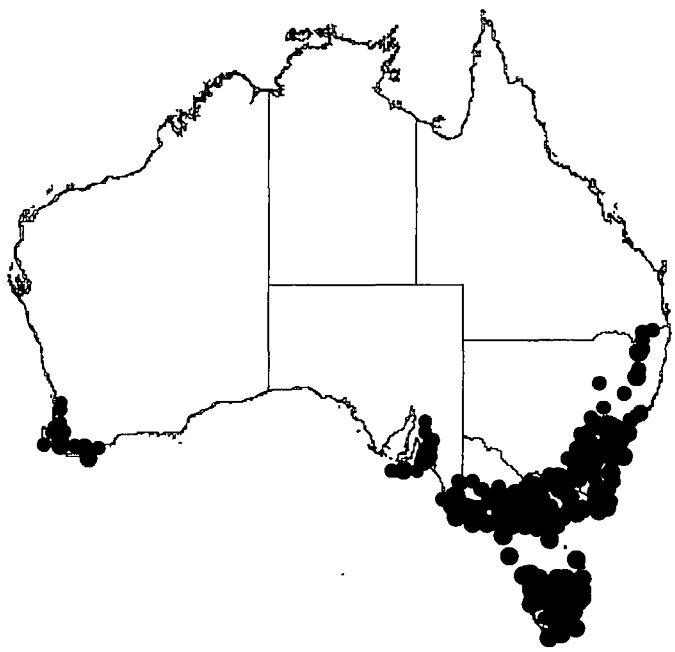


Figure 6.12 - Locations with similar climates to Scottsdale in Australia. Larger dots indicate higher MI's.

The linear regression of average defoliation level and EI during the first season shown in Figure 6.13 was not significant for the Tasmanian model (Spearman rank test: df = 41, $r^2 = 0.11$ p = 0.67). This suggests that the model parameters did not accurately explain the distribution of *A. orphana* and factors not accounted for in the model were affecting distribution. Adjusting the parameters when the mainland data was included did not improve the model further.

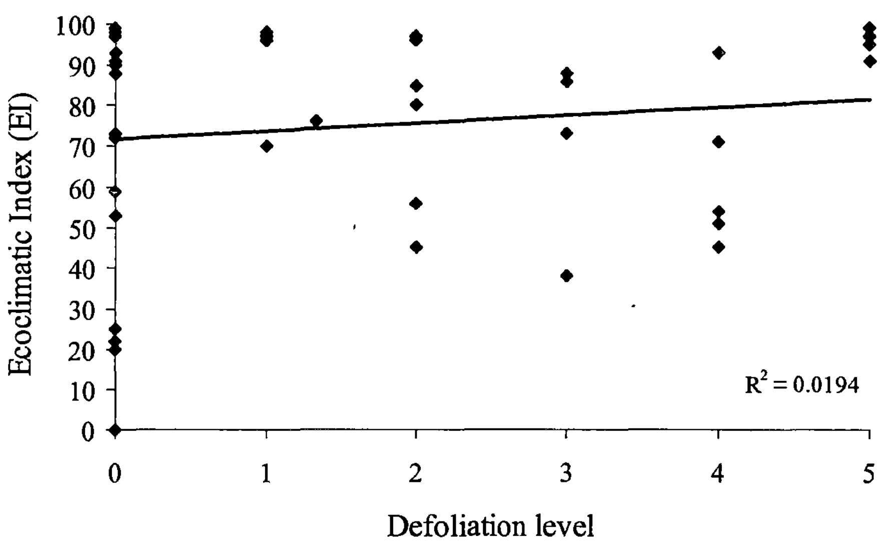


Figure 6.13 - Average defoliation level and ecoclimatic index for Tasmanian sites. (A zero value denotes no insects present in the survey)

The model developed from the survey data was, however, considered to describe the potential distribution of the insect more accurately than the match climates function, as it allows for variation in environmental stresses as well as rainfall and temperature which can affect the ability of an insect to populate an area. For this reason, it was decided to apply the model of the known distribution to the remainder of the world. Four more maps showing the potential distribution were created; Africa and eastern Europe (Figure 6.14), America and surrounding countries (Figure 6.15), New Zealand (Figure 6.16) and Asia (Figure 6.17). These predictions and the comments made are based on the assumption that the insect would establish if appropriate hosts were present.

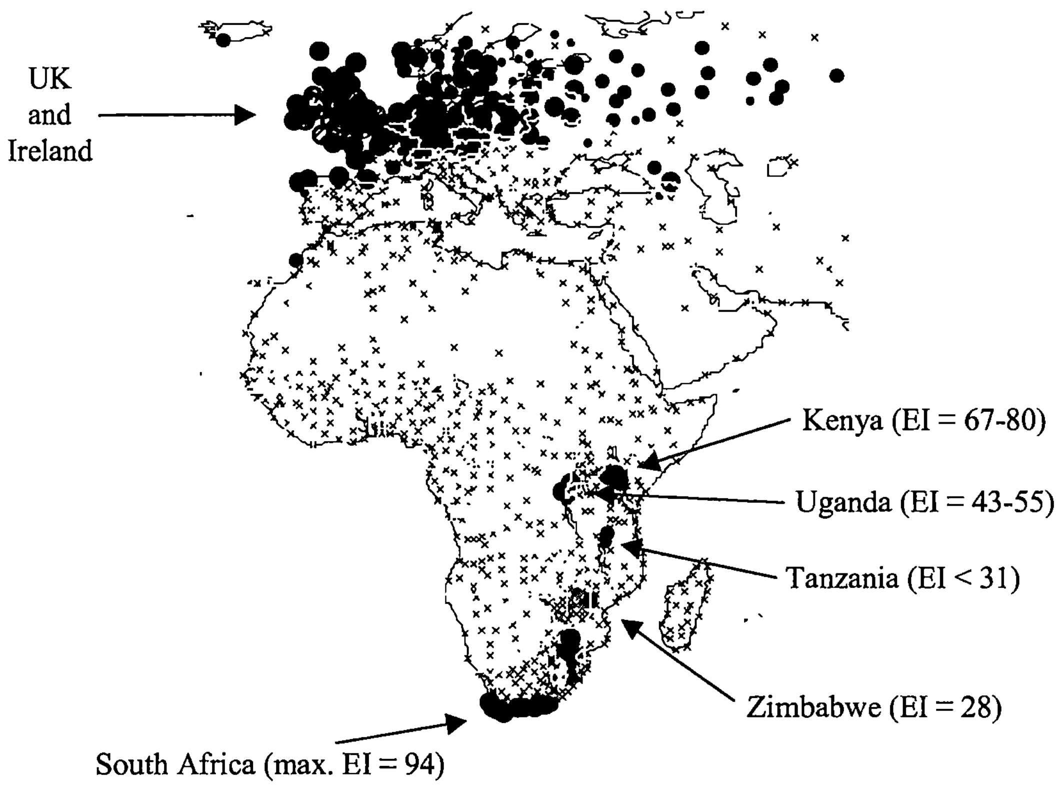


Figure 6.14 – CLIMEX model predicting the distribution of *A. orphana* in Africa and Europe. Larger dots indicate higher EI's.

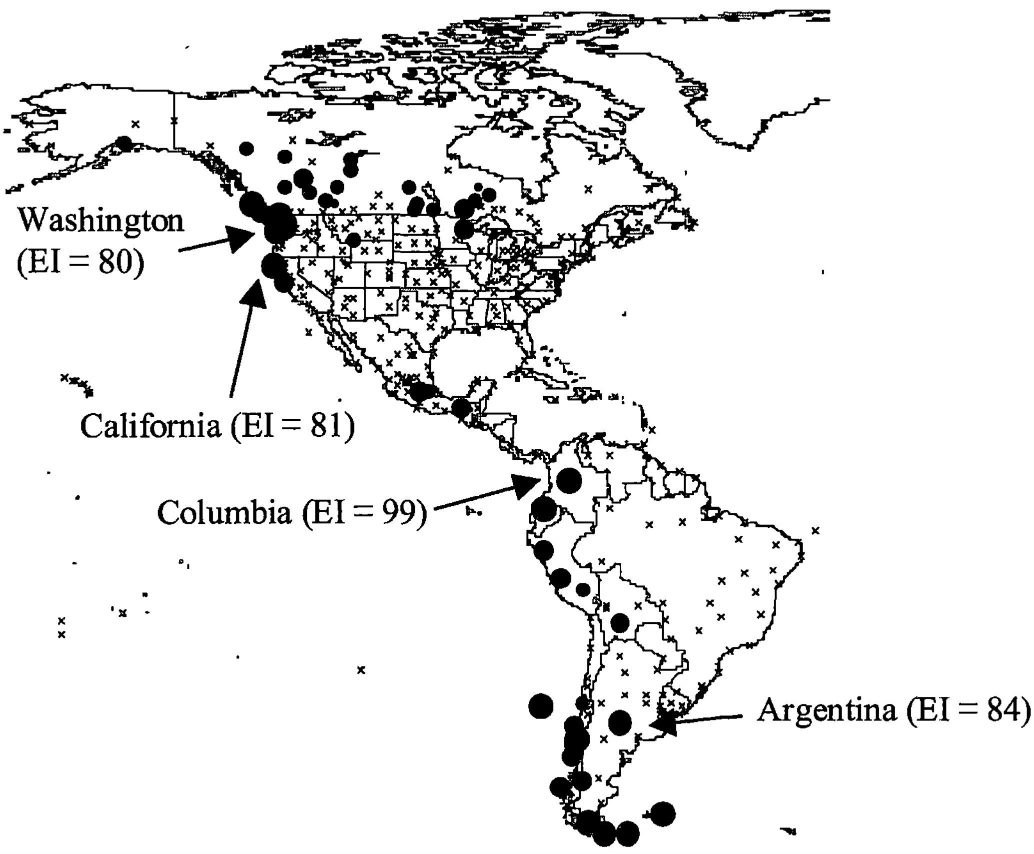


Figure 6.15 – CLIMEX prediction of locations where *A. orphana* has potential to establish in America. Larger dots indicate higher ecoclimatic index values.

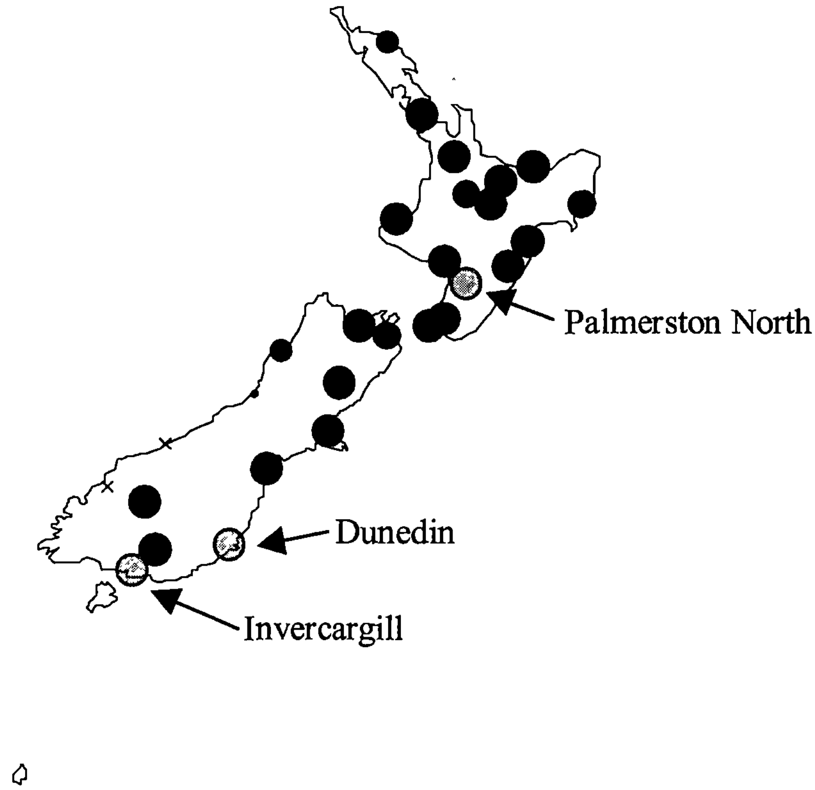


Figure 6.16 – CLIMEX prediction of locations where *A. orphana* would establish in New Zealand. Larger dots indicate higher ecoclimatic index values.

Shaded dots indicate EI = 100.

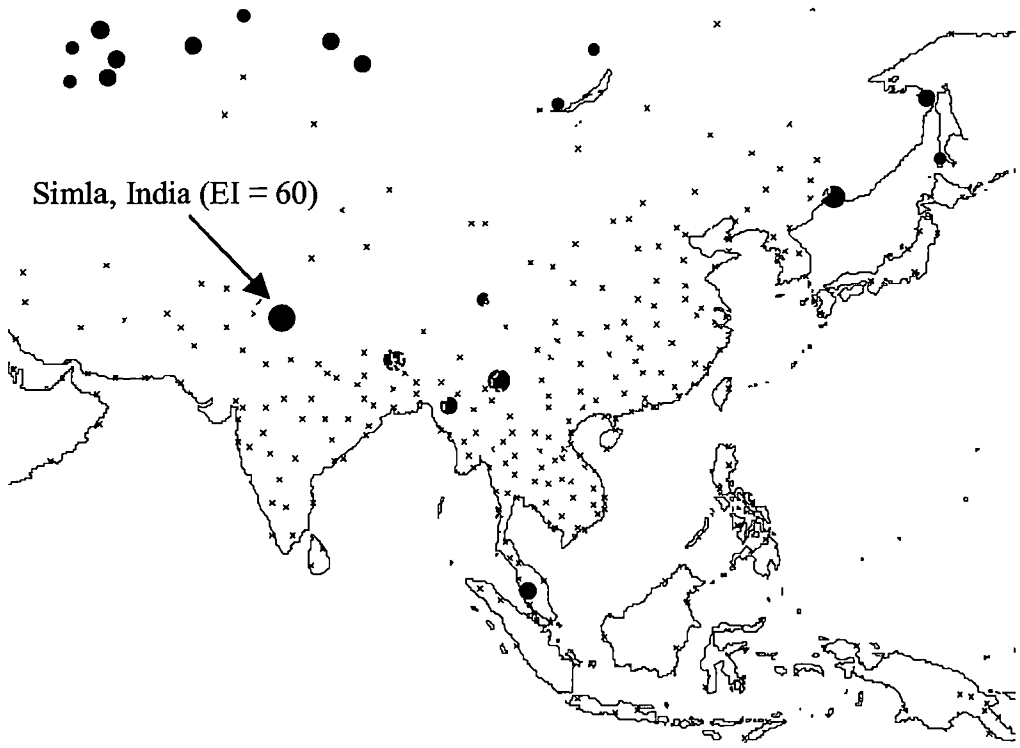


Figure 6.17 -CLIMEX prediction of locations where *A. orphana* would establish in Asia. Larger dots indicate higher ecoclimatic index values.

In Figure 6.14, the main areas where the model suggests that *A. orphana* has potential to become established are in the UK, Ireland and South Africa. Locations such as Cork in Ireland and Scilly Isles in the UK have EI values of 100. In the UK, the only area (from those for which climatic data was available) that did not have an ecoclimatic index greater than 70 was Greenwich. This indicates that *A. orphana* would be able to establish, but perhaps not as well as in areas where the EI values were greater. In Africa, the model predicts *A. orphana* has potential to establish in several locations, but high EI values are found only in Kenya and South Africa. Locations such as Morocco, Uganda, Tanzania and Zimbabwe also recorded EI values, but these were all less than 55, suggesting the climate in these areas was less than optimal for population establishment.

In North America the insect has potential to establish in North Head, Washington and Eureka, California (Figure 6.15). Canada has some locations with EI values up to 70, but cold stress reduces many of the other locations in North America to EI values of 55 or lower. In South America, the model shows that *A. orphana* has potential to establish in several locations along the east coast. The highest EI for this continent is observed in Bogota, Columbia (EI = 99). Several locations in Chile have EI values of up to 78. The main factors inhibiting potential establishment in South America are dry stress or wet stress, depending on the area.

In New Zealand (Figure 6.16), several locations show potential for *A. orphana* to establish. In particular, Dunedin and Invercargill on the south island and Palmerston North on the north island all had EI values of 100. The diapause parameters in the model were responsible for inhibiting the distribution in regions where the EI was lower, and hence further improvement in this part of the model would enable more accurate predictions of the potential of the Fireblight beetle to establish in New Zealand, Australia's closest neighbour.

CLIMEX predictions for Asia show the highest EI of 60 at Simla, India (Figure 6.17). Other locations in India, China and Malaysia have EI values < 51. The main limiting factors for establishment in India are moisture, heat stress and the parameters affecting diapause. The EI values for Russia are all less than 30.

6.4 Discussion

6.4.1 Geographical distribution of **A. orphana** *in SE Australia*

The geographical distribution of *A. orphana* and its host trees *A. dealbata* and *A. mearnsii* has been extensively surveyed during two seasons in Tasmania. The distribution of the insect was also determined on the mainland during a brief distribution survey and through examination of collection labels. During this exercise the benefit of museum collections was highlighted, as museum specimens collected in New South Wales provided points extending the distribution which would not have otherwise been obtained.

In Tasmania *A. orphana* was found in approximately equal incidence on both hosts, however greater numbers of *A. dealbata* were present in the state compared to *A. mearnsii.* On the mainland, whilst actual numbers of each host species were not evaluated, the situation appeared to be reversed, with *A. mearnsii* being more common. This may suggest that both hosts are an acceptable food source and that the insects do not discriminate between hosts when searching. The single observations of the *A. orphana* on other bipinnate species, *A. decurrens* and *A. baileyana* suggests that it may also feed on these species, possibly if the other species are not available or have already been defoliated. Similar observations have been made by researchers working with Bruchid beetles, *Stator limbatus* and *S. pruininus* (Siemens *et al.*, 1991) and the Colorado potato beetle, *Leptinotarsa decemlineata*, which uses Horsenettle, *Solanum carolinense* as an alternate host (Mena-covarrubias *et al.*, 1996). The development of *A. orphana* on the two different hosts, *A. dealbata* and *A. mearnsii* is explored further in Chapter 7.

A comparison of the incidence of *A. orphana* during the second season (1997-98) between the mainland and Tasmania showed beetles were present at slightly more sites in Tasmania (71% compared to 63%). The first season however, incidence was considerably less in Tasmania (33%). The differences between seasons may be due partly to differences in seasonal climatic effects, as climatic averages during the study (Bureau of Meteorology, www page) showed that regions where the insect was common in southeastern Australia experienced below average rainfall. Thus, the effects of drought stress on the trees could have been accumulating, making them more susceptible to insect damage prior to and during the second season surveys. White (1969) applied a stress index to water stressed *E. fasciculosa* and found that a high index value was strongly correlated with psyllid outbreaks. More recently, this relationship has been termed the 'plant stress hypothesis' (Preszler and Price, 1995). Furthermore, Tasmanian climatic data for the two years surveyed showed that Tasmania had below average rainfall in the north- and central-eastern regions. It is possible that the severely defoliated trees in these regions may have been infested with similar numbers of insects as the less defoliated trees, but were less able to replace lost foliage due to moisture stress. This has been observed during herbivory studies in *Eucalyptus camaldulensis* (Stone and Bacon, 1995). The results of population size estimates for the first season however, suggest that this did not occur with *A. orphana* feeding on *A. dealbata*. Population estimates were not evaluated during the second season when the trees may have been under more stress due to two years of less-than-average rainfall.

Few records of *A. orphana* were obtained from NSW, yet both host species extend north through this state indicating that the host distribution is greater than that of the pest. This difference in geographical distribution appears to be related to the differences in the maximum temperature preferences of the insect and its hosts. *A. mearnsii* and *A. dealbata* grow at temperatures up to 40 °C (Boland *et al.*, 1992) whilst the maximum temperature estimated for *A. orphana* in Chapter 4 was much lower, at approximately 25 °C. Visual comparison of the geographical distribution of the insect with temperature maps (Bureau of Meteorology, www page) suggests that the current distribution of the insect is closely linked to the regions where the average annual maximum temperature is 23 °C or less and the minimum annual temperatures are approximately 1 - 6 °C. Rainfall in these areas is relatively consistent throughout the year and but ranges between annual minimum and maximum values of approximately 400 and 1000mm.

Topography and altitude over the examined range of 10 – 800 m ASL do not appear to have any effect on the distribution of the *A. orphana*. It is possible that soil type may have an effect on host species and therefore insect distribution, as the nutrient-poor quartz soils of western Tasmania (pers. obs.) were home to few host species and insects compared to the rest of the state.

The geographical distribution of *A. orphana* surveyed at set locations both seasons showed a net increase in infestation of approximately 26%. Collection of geographical distribution data over a longer period of time would provide more information on the movement and population changes in this insect. However, the current observations suggest that a site defoliated in the first year was likely to be defoliated in the following year and the level of defoliation would be greater.

6.4.2 Modelling the geographical distribution of A. orphana

The geographical distribution of *A. orphana* in Tasmania was modelled using CLIMEX. This model was based on the known distribution of *A. orphana* in Tasmania. Application of the model to the southeastern part of mainland Australia showed climatic conditions that support *A. orphana* in several locations, including locations where the insects were not observed during the field survey. The lower developmental threshold of 1.6 °C estimated for *A. orphana* in the model is lower than predicted from the laboratory data in Chapter 4, but similar to that estimated from the field phenology studies. This again supports the observation that laboratory studies do not accurately predict the field development times of this particular insect.

The application of the model to the remainder of the world showed several locations where the beetles could establish if introduced. Plantations of *Acacia* have been established in several African and Asian countries, including Thailand (Wichiennopparat *et al.*, 1998), China (Fangqui *et al.*, 1998, Haojie *et al.*, 1998), Vietnam (Thinh, *et al.*, 1998), Zimbabwe (Muneri, 1997), South Africa (Chaunbi, 1997), Tanzania and Kenya (Boland, 1997). In these countries *A. mearnsii* is used for tannin production as well as for domestic fuel and building materials. *Acacia* pulp is also being investigated for paper making (Turnbull *et al.*, 1998). Accidental introduction and establishment of *A. orphana* in any of these countries may result in severe economic losses should the insect establish itself and become a pest. Evaluation of EI values for locations in Kenya such as Eldoret and Thika, where *Acacia* plantations are currently established all showed EI values of zero. Similarly, Njombe in Tanzania and Chipinga in Zimbabwe also showed zero values. In all these areas, the potential of *A. orphana* to establish a population was restricted by heat and/or dry stresses and thus it is unlikely that the current plantation areas would be infested by *A. orphana* should it be introduced to Africa.

Another country where the insect has potential to become a pest is New Zealand, which recorded high EI values in several locations. Whilst *A. orphana* is not present in New Zealand yet, the predictive model suggests that it could become established and thus provides an early warning that much of the NZ climate is acceptable for population growth. Any containment procedures will need to be implemented quickly to prevent spread if the insect is identified in this country.

The comparison of EI to known defoliation levels is a useful method of determining accuracy of the model and in this instance showed that the model did not accurately explain the observed distribution, based on defoliation. Using the match climates function in CLIMEX was much simpler than attempting to model a known distribution and has been used to predict the potential distribution of terrestrial planarians in the UK by Boag *et al.* (1995). The prediction obtained using this methodology may not be as accurate as a model based on a known distribution however, as it does not take into consideration the environmental stresses affecting the insects. That relatively high MIs were obtained for several areas where *A. orphana* was not present in Tasmania and Victoria also shows that other factors may be affecting its geographical distribution. These factors could be expected to be the same ones that limit the accuracy of the CLIMEX model based on the known distribution. One hypothesis may be that soil type affects host tree growth and hence insect distribution. Alternatively, host tree prevalence or genotype may affect the distribution of the beetle and cannot be accounted for by the model. If *A. orphana* was established in other countries and there was a larger known geographic distribution to base the model on, it would be possible to further improve the model's accuracy.

6.4.3 Implications for **Acacia** *forestry in SE Australia*

Acacicola orphana is capable of causing considerable losses in plantation productivity through severe and repeated defoliation of host trees. It does not appear to favour either of *A. dealbata* or *A. mearnsii* (as measured by average infestation levels) in southeastern Australia and in the survey up to 71 % of sites were infested. Although only two seasons were sampled, the assessment of defoliation showed that approximately half of all infested trees will be heavily defoliated (level 4 and 5 defoliation), with some green bark removal and most foliage chewed. The bark-feeding behaviour is unusual in a leaf-feeding insect and is of particular importance because of the potential of *A. orphana* to ring-bark the trees. In particular young trees are easily ring-barked and have few reserves for recovery. Bark feeding behaviour will be addressed further in Chapter 8.

The relationship between host tree size and defoliation by *A. orphana* was not significant, although larger, older trees may have a greater chance of survival after a severe defoliation event due to more reserves for recovery than a younger tree. Strauss and Morrow (1988) found that plant height was a good indicator of *Chrysophtharta hectica* (Boisduval) beetle numbers in *Eucalyptus* hosts, however this was not observed in this study.

In summary, the CLIMEX model shows that *A. orphana* has potential to further extend its distribution in Tasmania and throughout the world. Due to the high potential for *A. dealbata* and *A. mearnsii* to be severely affected by this insect, further research concentrating on the host species and determination of less susceptible trees would be beneficial to reduce potential losses in plantations. Preliminary studies involving *A. dealbata* provenances and the pest are presented in Chapter 9.

7. Acacicola orphana and host choice – Acacia dealbata or Acacia mearnsii?

Abstract

The host choice of *A orphana* between *A. mearnsii* and *A. dealbata* was examined using field observations, choice and no-choice oviposition laboratory studies and a larval development study. Field observations showed that where both *A. mearnsii* and *A. dealbata* were present in a location, they were equally likely to be infested. Development was 27% faster when fed a diet of *A. mearnsii*, and pupae were also significantly heavier. Oviposition was not significantly different between different hosts and there was no oviposition preference for the host from which the female was collected. Oviposition appeared to be affected by the cages, as females were located on foliage less than 50% of the duration of the experiment.

7.1 Introduction

7.1.1 Host choice, oviposition preference and larval performance

Host location and selection requires foraging by the insect and may be affected by visual or chemical cues (Beck, 1965; Bach, 1980; New, 1988; Bernays and Chapman, 1994; Tomlin and Borden, 1996) as were discussed in detail in Chapter 2, Section 2.4.2. Once a host has been selected, chemical stimuli may regulate oviposition (Beck, 1965; Hsiao, 1969). It makes sense for ovipositing females to discriminate between hosts, selecting for those that will maximise larval survival, growth and development (hereafter referred to as performance). This has been shown by Carr *et al.* (1998) in their study on the sawfly, *Nematus oligospilus* Forester which laid eggs on the more vigorous shoots, thus promoting better larval performance and a shorter development time. The example of *Spodoptera exigua* provided in Section 2.4.2 shows that oviposition preference does not always correlate positively with offspring performance, as the females of this species oviposited on a host that did not provide optimum larval performance (Berdegue *et al.*, 1998). This finding is supported by studies of Pires *et al.* (2000) on the tropical

Spittlebug *Deois flavopicta* Stål and Craig *et al.* (2000) who worked with the fly, *Eurosta solidaginis* (Smith). Hsaio (1969) suggested the discrepancies between host choice for oviposition and larval performance may occur when the stimulants for oviposition and feeding are not the same. More recently, in a review of oviposition preference and larval performance (where performance refers to larval growth, development and survival) Thompson (1988) provides four hypotheses to explain the differences in oviposition preference and performance, these are explained and related to *A. orphana* below;

1.Time hypothesis – A new plant in an area is laid on. In time, females may learn not to lay on this plant or alternatively, the larvae may adapt to the plant. A discrepancy in oviposition preference/larval performance results may occur if there was inadequate time for the evolutionary response to occur. Also, the rate of evolution of oviposition preference may be different to that of larval performance. This hypothesis may apply to *A. orphana.* However it is unlikely. Both *A. dealbata* and *A. mearnsii* are native to Tasmania and thus inadequate time for the evolution of oviposition preferences and larval performance seems doubtful.

2. Patch dynamics hypothesis – A more common host may receive more eggs than a less common host because it is more apparent in the environment and is encountered more frequently. If this occurs, variation in plant structure and community composition is affecting oviposition preference/larval performance. This may occur with *A. orphana* on *A. dealbata* in Tasmania (and on *A. mearnsii* on mainland Australia) where one host is considerably more common than the other (see Chapter 6) giving rise to the observation that *A. orphana* has a preference for *A. dealbata* in Tasmania and *vice versa.*

3. Grazer/Parasite Hypothesis – A parasite is representative of larvae that do not move between hosts during development whilst a grazer can feed on many hosts or many parts of a host, including those that may not be selected for oviposition. Thus, larval feeding studies require an understanding of the 'normal' diet of an insect before comparing results with oviposition preference studies. *A. orphana* feeds predominantly on the foliage and green bark of the host on

which oviposition occurred. However, studies in Chapter 8 show that green bark of *A. dealbata* does not enhance growth of *A. orphana* over larvae fed only foliage. Grazing, in the case of *A. orphana* would therefore seem unlikely to explain any discrepancy in oviposition preferences in relation to larval development.

4. Enemy free space – Experiments where enemies are not present may yield different results for oviposition preference and larval performance due to factors such as natural mortality. In the study here, larval development and mortality were evaluated in the laboratory, in the absence of natural enemies. Thus, the experimental design does not allow for the testing of this hypothesis.

7.1.2 Nutrition

The effects of leaf chemistry on feeding were also discussed in detail in Chapter 2 (Section 2.4.2). In particular, foliar nitrogen content has been positively related to paropsine larval development (Fox and Macauley, 1977, Morrow and Fox, 1980; Ohmart *et al.*, 1987). Leaf toughness has been negatively correlated to nitrogen content, and thus larval feeding is affected as larvae are either not able to feed on the tougher foliage or must consume more to meet nutritional requirements (Ohmart *et al.*, 1987). Moisture content of foliage also decreases with age, and this may affect feeding or oviposition. Pires *et al.* (2000) found a positive relationship between females of *Deois flavopicta* Stål and oviposition in moister pots containing host plants (this insect does not oviposit directly on to plant tissue). Scriber and Slansky (1981) suggest foliar moisture levels above 70% are optimal for larval development. As *Acacia* species are nitrogen-fixing legumes it is unlikely that a lack of nitrogen would affect feeding of *A. orphana*. However, it is not known what level of nitrogen is present in different species of *Acacia* or how it affects insect feeding or oviposition.

7.1.3 Paropsine host interactions

Of the paropsines recorded feeding on *Eucalyptus*, many have been observed to have a wide host range and whilst a single species can feed and develop on a variety of hosts, it may be more frequently observed on some hosts than others. *A. orphana* has been located predominantly on *A. dealbata* and *A. mearnsii*, but also on other species as outlined in Table 7.1. Personal observations of the larvae feeding on *A. baileyana* and adults feeding on *A. decurrens* in the field were recorded during the study in Chapter 6. Adults were also observed feeding on juvenile *A. melanoxylon* foliage in the glasshouse in February 1997.

Table 7.1 - Trees from which *A. orphana* has been recorded feeding. Superscripts: L = larval feeding, A = adult feeding, ? = lifestage not noted.

Host species	Authors
A. dealbata	French, 1911^{L}; Elliott, 1978^{L,A}; Van den berg, 1982^{A}; Elliott and de Little, 1985^{L,A}; Bashford, 1990$^{?}$; Bashford, 1991$^{?}$; this study^{L,A}.
A. mearnsii	*Elliott, 1978^{L,A}; Van den berg, 1982^{A}; Elliott and de Little, 1985^{L,A}.
A. decurrens	French, 1911^{L}; this study^{A}.
A. verticillata	Bashford, 1990$^{?}$.
A. saligna	Van den berg, 1980^{A}.
A. melanoxylon	This study^{A}.
A. baileyana	This study^{L}.
A. longifolia	Van den berg, 1982^{L,A}.
Acacia / Wattle	Froggatt, 1923^{L}; McKeown, 1942^{L}.

* incorrectly identified as *A. molissima* Willd.

Interactions between *A. orphana* and its hosts have not been documented in detail. Larval development and survival of *Eucalyptus* feeding paropsines has been shown to vary according to host species. For example, *P. atomaria* larvae matured faster when fed on *E. blakelyi* compared to *E. maculosa* (Carne, 1966b). Larvae of *C. bimaculata* developed faster and consumed less *E. nitens* foliage compared to *E. regnans* foliage (S.C. Baker, J.A.Elek and S.G. Candy, unpublished data). They found that efficiency of conversion of the *E. nitens* foliage was almost double that of *E. regnans*, suggesting that *E. nitens* was a better food source. Observations of de Little and Madden (1975) outlined in Chapter 2 suggest that *C. bimaculata* and *C. agricola* have adapted to different hosts in the field. One explanation for this adaptation may be to avoid resource competition, although this has not been studied. Oviposition preferences (Hanks *et al.*, 1995a), host apparency (Thompson, 1988) and colour (Prokopy and Owens, 1983; Raymond, 1998) may also be important in the host selection process which was detailed in Chapter 2, section 2.4.

7.1.4 Experimental design and host selection

It is possible to examine host selection in the field, or in either choice or no-choice experiments in the laboratory. Field studies provide an environment in which the ovipositing females are not affected by the conditions of the experiment (ie. no cage, no handling, unrestricted movement). However, there are many uncontrollable abiotic and biotic external influences (including the 'condition' of the females). Laboratory experiments provide an environment where choices of females can be better manipulated but, there may be caging effects (Withers and Barton Browne, 1998). In a cage, many of the sensory cues an insect would encounter in field conditions may be absent and therefore affect oviposition (Withers and Barton Browne, 1998). Other caging effects may result in random oviposition, where eggs are laid on all available surfaces including the cage (Stange, 1997). Alternatively, some eggs could be laid on the expected target host, but more on a less- or non-expected host (Purcell *et al.*, 1997). Withers and Barton Browne (1998) also observed that an insect in a cage might give higher priority to escaping than oviposition, a behaviour that is difficult to overcome. One further behaviour is that of indiscriminate oviposition, where a female is extremely ready to oviposit, and is therefore less selective in the choice of oviposition substrate (Withers and Barton Browne, 1998).

7.1.5 Chapter aims

During the geographical distribution study in Chapter 6, *A. orphana* was observed on both *A. dealbata* and *A. mearnsii*. *A. dealbata* was the dominant tree species in Tasmania and *A. mearnsii* appeared more prevalent on the mainland. The Tasmanian studies (in Chapter 6) showed that *A. orphana* fed approximately equally on both hosts in Tasmania. In a few areas where *A. dealbata* and *A. mearnsii* were both present however, visual observations suggested that defoliation of *A. mearnsii* commenced only after nearby *A. dealbata* was already mostly defoliated. Thus, to examine the host choice of *A. orphana*, the following points were investigated.

1. Infestation levels of the trees when both species were closely positioned in the field.

2. Larval mortality, growth and development on *A. dealbata* and *A. mearnsii* in the laboratory.

3. Oviposition choice between *A. dealbata and A. mearnsii* in the laboratory.

7.2 Materials and Methods

7.2.1 Infestation of **A. mearnsii** *and* **A. dealbata** *in Tasmania*

To examine if *A. orphana* caused more defoliation on one host or the other in the field, locations where both hosts were present were identified during October 1998 (Figure 7.1).

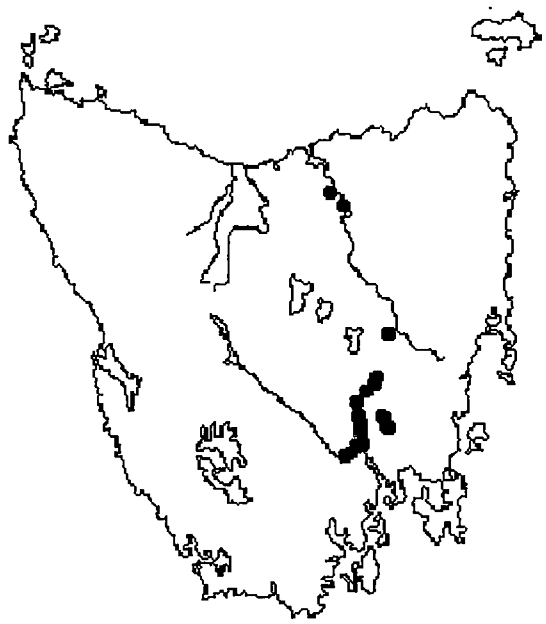


Figure 7.1 - Locations where *A. dealbata* and *A. mearnsii* were both observed in Tasmania.

At each location, the presence or absence of *A. orphana* larvae was noted and a percentage defoliation score allocated to each tree species. If only one tree of each species was present, then the score was based on only one, if more trees of each species were present, then the score was an average for the 'group'. A 'group' consisted of 2-10 trees. In the scoring system, 100 was total defoliation and 0 denoted no damage. Within this range, there were seven score categories as defined in Table 7.2.

Table 7.2 - Defoliation scores and descriptions

Score	Description
0	No damage.
5	Minimal damage.
25	>5% but <50% damage, about ¼ of canopy defoliated.
50	Half of canopy defoliated.
75	>50% but < 100% damage, about ¾ of canopy defoliated. Some bark chewed.
95	Most of canopy removed, few leaves remaining. Bark chewed.
100	Canopy totally defoliated, no green foliage remaining, tree dead in appearance, bark chewed.

The distance between the closest *A. dealbata* and *A. mearnsii* tree was also measured at each location. The study was conducted using damage scores as an indication of infestation rather than egg counts, as it was too difficult and time consuming to search the trees for eggs due to the number of trees at many locations and the large size of some of the trees. It is recognised that the damage score may not truly reflect oviposition due to mortality in the larval stages (which may differ between the two hosts).

A preliminary examination of foliar colour of the two different hosts was also performed on a subset of 6 trees of each species from a single location, using a Minolta CR-200b Chromo Meter (Minolta, Japan). Prior to measurement, foliar samples collected were oven dried at 75 °C for 72 hours and ground in a hammer mill to ensure uniformity of the samples. The Chromo Meter measures the reflected light of the samples in L*, a* and b* co-ordinates. L* is a measure of lightness, where 100 is white and 0 is black. a* and b* are measures of chromaticity, where if a* > b* then the sample is more red and if b* > a*, then the sample is more brown (Baldois and

Banks, 1975). The three co-ordinates can also be combined to provide a single colour difference value (ΔE) using Equation 1.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Equation 1}$$

7.2.2 Effect of host on larval survival, development and growth.

To investigate any effect of host species on larval survival, development and growth, adults were collected from *A. dealbata* trees at Buckland (42° 39'S, 147° 36'E) in April 1998 and placed in a cage in the laboratory. After 24 hours all eggs were collected from the foliage and groups of 10 eggs were randomly placed in each of 32 plastic Petri dishes (9 cm diameter) with moistened filter paper in the base to reduce desiccation. These were incubated in Contherm CAT 150 MCP cooled incubators at 17 °C ± 1 °C; 8L:16D; ~66% R.H. These conditions were found appropriate for development in the studies in Chapter 4.

Upon hatching, larvae were fed a diet of either *A. dealbata* or *A. mearnsii* foliage from a single tree of each species. A single tree of each host species was used to provide the foliage as insects had been found to feed and develop successfully on these particular trees in pilot studies. Using the same foliage also reduced variation in development due to different hosts, although how representative these trees were of each species remains untested. Abundant fresh food from these trees was provided three times weekly when the containers were checked. There were 16 replicates for each host tree. The number of insects of each stage present when the containers were checked was noted and this enabled an average developmental time for each stage, in each container to be determined (individual insects were not tracked for development).

Mortality was calculated for each larval stage feeding on each host species. Development time in days was also calculated. Any larvae that reached the pupal stage were weighed 48 hours after pupation. To satisfy the normality assumption of the analysis of variance used, results for the first instar were transformed alogarithmically, whilst a log transformation was applied to the second and third instar results. No analysis was conducted for the pupal or adult stages due to insufficient data.

Nitrogen content of the foliage of each host species was analysed using the wet-digestion method outlined by Lowther (1980). This involved drying the foliar material at 75 °C for 72 hours and grinding it in a hammer mill. Approximately 0.016g of re-dried, ground material was digested in 4 ml of concentrated sulfuric acid (H_2SO_4), and 2 ml of hydrogen peroxide (H_2O_2) (30% w/v) at 360 °C for 30 minutes. After cooling to 150 °C, hydrogen peroxide was added dropwise until the solution cleared to a pale yellow colour. The samples were then digested at 360 °C for a further hour, resulting in a clear digestate. This was diluted and colourmetrically analysed for nitrogen (QuikChem method 10-107-06-2E, Lachat Instruments, Wisconsin) on a flow injection analyser (QuikChem 800, Lachat Instruments, Wisconsin).

Moisture content was assessed as a percentage of the wet foliage weight. This involved weighing the fresh (wet) foliage, oven drying it for 72 hours and then re-weighing. The difference between the wet and dry weights was then divided by the wet weight and multiplied by 100.

Two methods were used to measure leaf toughness: a leaf penetrometer (Sands and Brancatini, 1991) and the specific leaf weight method, by Landsberg and Gillieson (1995).

7.2.3 Oviposition behaviour in relation to host plant

The host choice of ovipositing females was observed in cages in a glasshouse in April 1999. Females were collected from *A. dealbata* at Conara (41° 50'S, 147° 26'E) and from *A. mearnsii* at Colebrook (42° 33'S, 147° 22'E). Cages measured 22 x 26 x 36 cm and were constructed of heavy, slightly opaque plastic with windows cut in four sides and covered with fine fabric mesh. In each cage, two leaves, similar in size and age-class were placed in small water filled vials approximately 20 cm apart, stuck to the upturned lid of the cage with velcro. To seal the cage, the lid was put flat on a bench, and the up-ended base put on top. The cages were exposed to natural light and photoperiod. Relative humidity was 55 % (range 30 – 91% diurnally) and temperatures were set to fluctuate diurnally between a minimum of 12 °C and a maximum of 20 °C.

Treatments were as follows;

5 cages with insects collected from *A. dealbata* provided with *A. dealbata* and *A. mearnsii*,
5 cages with insects collected from *A. dealbata* provided with only *A. dealbata*,
5 cages with insects collected from *A. dealbata* provided with only *A. mearnsii*,
5 cages with insects collected from *A. mearnsii* provided with *A. dealbata* and *A. mearnsii*,
5 cages with insects collected from *A. mearnsii* provided with only *A. dealbata*,
5 cages with insects collected from *A. mearnsii* provided with only *A. mearnsii*.

Three females that appeared gravid and thus ready to oviposit were placed equidistant between the two vials in each cage according to the treatments above. These were selected and separated from males by their obviously distended abdomens.

After 48 hours the insects were removed and dissected to confirm sex. The number of eggs on the foliage in each vial was then counted.

7.2.4 Oviposition behaviour in host choice trials

To further examine the host selection process of adult *A. orphana*, individual gravid females collected from *A. dealbata* in the field were placed in cages in the same environmental conditions used in section 7.2.3. In each cage, one vial contained a single leaf of *A. dealbata* and the other vial a single leaf of *A. mearnsii* (Figure 7.2). The leaves were all of similar age-class and size. At approximately hourly intervals between 9 am and 5 pm the location of the insect in each of the 30 cages was noted. At 9am and 5pm each day the foliage was checked for eggs. The study was conducted for two full days (17 observation periods).

Figure 7.2 - Cage set-up for oviposition study. A single leaf of *A. mearnsii* in vial on left and *A. dealbata* on right.

7.3 Results

7.3.1 Infestation of A. dealbata *and* A. mearnsii *host plants at the same field location*

A total of 19 sites where *A. dealbata* and *A. mearnsii* were both present were identified in Tasmania. The average distance between the closest *A. mearnsii* and *A. dealbata* trees at each site was 27.8 ± 16.0 m (mean ± SE, n = 19, range 1 - 300 m). Most locations were in the south-east of the state and none were in the western half of Tasmania (Figure 7.1). At only 2 sites was neither host species infested by *A. orphana*, whilst at 12 sites (63 %) both species were infested. Five locations had only one species infested - *A. dealbata* in three sites and *A. mearnsii* in the remaining two (Table 7.3).

Table 7.3 – Occurrence of *A. orphana* in *A. mearnsii* and *A. dealbata* at different sites.

Site Information	Count (n)	Proportion of total (%)	Distance apart (m) (Average ± SE)
No trees infested	2	10.5	6.5 ± 3.5
Both host species infested	12	63.2	39.9 ± 24.9
Only *A. mearnsii* infested	2	10.5	8.5 ± 1.5
Only *A. dealbata* infested	3	10.6	6.3 ± 5.3
Total *A. mearnsii* infested	14	73.7	
Total *A. dealbata* infested	15	78.9	

A pair-wise comparison of damage scores for each species at each site where both species were infested showed that one species did not experience significantly more damage than the other (Wilcoxon matched pairs: test statistic = 37.00, $n = 13$, $p = 0.59$). The relative numbers of each species and therefore apparency at each site was not quantified and thus, whilst one species may not have experienced significantly more defoliation than the other, it was not possible to examine damage in relation to apparency in detail. As a general observation however, *A. dealbata* was more prevalent than *A. mearnsii* at most of the locations. Average damage scores for the sites where both species were damaged are shown in Table 7.4. No significant relationship was observed for distance between the two species at each site and the difference in damage score (Regression; $F_{1,10} = 2.37$, $p = 0.154$). The distance between trees at sites where only one species was infested compared to sites where both species were infested was also not significantly different ($U = 23.0$, $n = 5,12$, $p = 0.46$). This result suggests that larval movement between trees is not responsible for spread of infestation, and that instead, females are ovipositing on all trees involved.

Table 7.4 – Damage scores (%), standard errors and range for sites where both *A. dealbata* and *A. mearnsii* were infested.

Host Species	Average damage (%) ± SE	Count (n)	Maximum	Minimum
A. dealbata	82.1 ± 8.16	12	100	10
A. mearnsii	70.0 ± 9.75	12	100	10

All foliar samples of both species were more brown than red in colour (ie. b* > a*). No significant differences were observed between the species for overall colour difference (ΔE: $F_{1,11} = 0.20$, $p = 0.674$), lightness (L*: $F_{1,11} = 0.10$, $p = 0.765$) or brown colour (b*: $F_{1,11} = 0.04$, $p = 0.848$). *A. dealbata* foliage was however, significantly less red than *A. mearnsii* foliage (a*: $F_{1,11} = 9.56$, $p = 0.027$).

Table 7.5 – Summary of colour co-ordinate values L*, a*, b* and ΔE for *A. dealbata* and *A. mearnsii*, averages, standard errors and range. n = 6.

Colour co-ordinate	*A. dealbata*	*A. mearnsii*
L*	50.4 ± 2.31	45.5 ± 1.84
	(44.6 – 60.0)	(42.6 – 54.7)
a*	-5.1 ± 1.20	-2.5 ± 1.17
	(-7 1 – 0.8)	(-5.8 – 2.6)
b*	26.9 ± 1.21	27.2 ± 0.87
	(24.7 – 32.6)	(24.5 – 30.1)
ΔE	56.7 ± 1.8	57.5 ± 1.61
	(48.3 – 61.0)	(51.6 – 62.7)

7.3.2 Effect of host on larval survival, development and growth

Mortality

Of the 320 eggs initially incubated, only 79% hatched. High mortality during the first instar reduced the numbers by a further 191, resulting in only 25% of emergent individuals reaching the second instar stage. Mortality was not patchy, but spread throughout the replicates, indicating consistency in the mortality agent. Larvae fed *A. mearnsii* foliage experienced less mortality than those fed *A. dealbata* in this experiment, but mortality on *A. mearnsii* here was comparable to that of larvae fed *A. dealbata* in the studies in chapter 4 (Table 7.6). No insects fed *A. dealbata* developed into adults. Only 30 individuals (24.2 % of hatchlings) fed with *A. mearnsii* reached adult stage.

Table 7.6 – Average overall mortality of *A. orphana* as a percentage of insects developing to that stage when fed *A. dealbata* or *A. mearnsii* and incubated at 17 °C, 8L:16D.

Stage	Host species		
	A. mearnsii Mean (n)	*A. dealbata* Mean (n)	*A. dealbata* – from Chapter 4 Mean (n)
Egg	22.5 (160)	19.4 (160)	4.5 (111)
First instar	54.0 (124)	96.1 (129)	45.3 (106)
Second instar	28.1 (57)	80 (5)	25.9 (58)
Third instar	4.9 (41)	100 (1)	37.2 (43)
Fourth instar	12.8 (38)	N/R*	7.4 (27)
Pre-pupa	0 (34)		16.0 (25)
Pupa	11.8 (32)		0 (21)
Adult	19.4 (30)		0 (21)
Overall mortality (%)	81.3	100	81.1

*N/R = no result due to 100% mortality.

Development

Development times for the different stages are shown in Table 7.7. Included in this table for comparison are the development times for insects at 17 °C fed with *A. dealbata* in Chapter 4. Overall, larvae fed with *A. mearnsii* developed into adults significantly faster ($F_{1,22} = 12.09$, $p = 0.003$) than those fed with *A. dealbata*. Significant differences were also observed between the different stages ($F_{5,22} = 8.13$, $p < 0.001$), and there was a significant interaction between the stages and diet ($F_{5,22} = 3.87$, $p < 0.05$). Development times for all stages except the first instar stage in the Chapter 4 values, were faster when insects were fed *A. mearnsii* foliage. The total development time from first instar to adults was approximately 25 % faster (26 days) for insects fed *A. mearnsii* when compared to insects fed *A. dealbata*.

Table 7.7 - Mean developmental time in days (± SE) and number of insects (in brackets) for each stage and treatment. All averages for columns with superscript 1 are based on n = 16 replicates.

Treatment	*A mearnsii*[1]	*A. dealbata*[1]	*A. dealbata**
First instar	22.4 ± 0.42	31.0 ± 1.38	17.0 ± 0.35
(n)	(57)	(5)	(58)
Second instar	8.8 ± 0.60	24	21.6 ± 0.45
(n)	(41)	(1)	(43)
Third instar	9.6 ± 1.47	N/R	14.0 ± 0.47
(n)	(38)		(27)
Fourth instar	10.8 ± 1.41		19.4 ± 0.37
(n)	(34)		(25)
Pre-pupa	7.8 ± 0.37		10.6 ± 0.41
(n)	(32)		(21)
Pupa	10.2 ± 1.38		14.0 ± 0.33
(n)	(32)		(21)
Total First Instar to Adult	70.5 ± 2.77		96.7 ± 1.45
(n)	(30)		(21)

*Developmental times and insect numbers from Chapter 4.; N/R = no result due to mortality.

The weights of pupae reared on *A. dealbata* in the study in Chapter 4 were significantly lower than those of larvae reared on *A. mearnsii* (Table 7.8) ($F_{1,27} = 4.61$, $p = 0.043$). However, sexes were not differentiated and thus if proportions of each sex were different in the samples there may be a bias in the results.

Table 7.8 - Pupal weights and range (mg) for larvae fed diets of *A. mearnsii* or *A. dealbata*.

Diet	Count	Average (mg)	Range (mg)
A. dealbata	15	10.4 ± 2.55	8.4 – 12.8
A. mearnsii	13	12.0 ± 3.86	7.3 – 16.5

The percent moisture in the *A. dealbata* foliage was 64.4 ± 0.40 (mean ± SE, n = 8) which was significantly higher than the value of 50.1 ± 0.20 (n = 9) obtained for *A. mearnsii* (Repeated measures; $F_{1,8} = 28.88$, $p < 0.005$). Nitrogen contents of the two different species were not significantly different (Repeated measures; $F_{1,5} = 0.52$, $p > 0.05$), with *A. dealbata* being 2.57% ± 0.174 (n = 6) compared to *A. mearnsii* which was 2.92% ± 0.223 (n = 6).

The two different methods used to measure leaf toughness were unsuccessful. The leaf penetrometer was unable to cleanly puncture the small pinnules of the foliage and despite attempts to make the point finer, pinnules still broke. The complex shape of the leaf and its overlapping pinnules meant that it was not possible to measure the leaf surface area accurately, and thus results from using the specific leaf weight method were also considered too inaccurate.

7.3.3 Oviposition of A. orphana *in choice and no-choice trials*

Of the 30 cages utilised, females laid no eggs in four, two of these were choice cages, and the other two were no-choice cages. In three cages, the females were collected from *A. dealbata* in the field, whilst in one the female was collected from *A. mearnsii*. When the females from these cages were dissected, ovarian status was the same as females from the other cages.

Overall, although beetles collected from *A. mearnsii* appeared more fecund on average, producing 18 % (61.9 eggs/♀) more eggs than those collected from *A. dealbata* (44.3 eggs/♀) this difference was not significant ($t = -0.319$, $p = 0.099$, $df = 28$) (Table 7.9). Also, if the choice and no-choice designs are compared, there was no significant difference in eggs laid per female between the two designs (20 eggs/♀ compared to 16.6 eggs/♀; $t = 0.71$, $p = 0.484$, $df = 28$).

In the no-choice design overall, the number of eggs laid by females collected from each of the two hosts was not significantly different ($t = -0.55$, $p = 0.592$, $df = 16$). Furthermore, females collected from one host species laid an equivalent number of eggs on either host species, suggesting that the host from which the female was collected did not limit oviposition on a different host ($U_{9,9} = 29.5$, $p = 0.32$). There was also no significant difference when comparing egg numbers laid on the host from which the females were not collected ($U_{5,5} = 12.0$, $p = 0.92$). No significant difference in the total number of eggs laid on the two lots of foliage provided within the cage existed (Wilcoxon matched pairs; test statistic = 45.5, $n = 18$, $p = 0.08$) (Table 7.9). Thus, the no-choice study showed no apparent discrimination between the host types by females due to the host from which they were collected and furthermore, whilst *A. mearnsii* received 33% more eggs in the no-choice design, this amount was not significant ($t = 1.35$, $df = 18$, $p = 0.195$).

In the choice design, females collected from *A. mearnsii* laid an equal number of eggs to those collected from *A. dealbata* (28.3 eggs/♀ compared to 17.3 eggs/♀; $t = 1.32$, $p = 0.259$, $df = 7$). Furthermore, irrespective of host collected upon, females laid an equal number of eggs on both host species provided (collected from *A. dealbata*: $U_{5,5} = 4.0$, $p = 0.07$; collected from *A. mearnsii*: $U_{5,5} = 12.0$, $p = 0.91$). Also, regardless of the host the adult females were collected upon, the number of eggs laid on each species in the choice trial was similar ($t = -0.98$, $df = 9$, p

= 0.353) (Table 7.9). Due to the small sample sizes, which may be affecting the significance of the results, the preceding analyses need to be repeated with larger sample sizes. Several females were also found walking about the edges of the cages or inactive in the crevice between the lid and the walls and thus larger cages may also be necessary.

Table 7.9 - Eggs laid by *A. orphana* on *A. dealbata* and *A. mearnsii* in choice and no choice situations. Three females per cage.

Host collected from	Oviposition substrate	Total eggs	Average eggs/cage ± SE (n)	Range/Cage
No-choice Study				
A. dealbata	*A. dealbata*	166	27.6 ± 16.66 (3)	0 – 78
A. dealbata	*A. mearnsii*	243	48.6 ± 11.31 (5)	24 – 81
A. mearnsii	*A. dealbata*	237	47.4 ± 14.09 (5)	1 – 78
A. mearnsii	*A. mearnsii*	352	70.4 ± 16.68 (5)	18 – 114
Choice study				
A. dealbata	*A. dealbata*	207	41.4 ± 19.12 (4)	0 – 102
	A. mearnsii	53	10.6 ± 7.26 (4)	0 – 27
A. mearnsii	*A. dealbata*	178	44.5 ± 18.75 (4)	0 – 86
	A. mearnsii	162	40.5 ± 22.01 (4)	0 – 109
Overall summary				
A. dealbata		669	55.8 ± 8.05 (12)	0 – 102
A. mearnsii		929	66.4 ± 6.08 (14)	0 – 109

7.3.4 Oviposition behaviour in host choice trials

Feeding damage was not apparent in all cages, suggesting that prior handling or another unknown variable may have affected some beetles. In nine cages (30% of all cages), females were not observed on either foliage type during the entire experiment. For simplification, the results have been divided into day 1 and day 2 in Table 7.10. The proportion of time spent on hosts by insects was calculated as a proportion of observation periods, with one observation point for each hour. On the first day, 63% of insects did not alight on any foliage. This decreased to 43% on the second day. On both days, females did not spend significantly more time on any one host species (day 1: $t = 1.00$, $p = 0.351$, $df = 7$; day 2: $t = -0.29$, $p = 0.780$, $df = 12$). No insects were recorded moving from *A. mearnsii* to *A. dealbata* during the daylight hours, however three females moved during the night period when regular observations were not made. In general, once insects alighted on foliage they tended to stay there and moved little. It is possible that the small sample sizes and late timing of the experiment in the field cycle of *A. orphana* may have biased the results.

A total of 147 eggs were laid by seven females. Of these, 55 were laid on *A. dealbata* and 92 on *A. mearnsii* (Table 7.11). A further breakdown of behaviour and egg counts is presented in Table 7.12. From this, it is apparent that the majority of females that laid (71.4%) did not visit both foliage types whilst 55.6% of females that did sample both foliage types did not lay at all. The egg counts in this study were less than observed in the previous section and approximately half the average egg numbers observed in chapter 3. This may be due the late timing of the study which meant females were collected late in the field season may have been nearing the end of their oviposition period.

Table 7.10 - Movement between hosts and time spent on hosts in cages during 15 observational periods. One female only per cage.

Day 1 - Host combination	Number of insects	Total no. of observations on host	Proportion of observational periods (%)	Range*
On no host	19	n/a		
On *A. mearnsii* only	6	12	29.0	14.3 – 57.1
On *A. dealbata* only	3	9	42.9	28.6 – 71.4
Moving from *A mearnsii* → *A. dealbata*	0			
Moving from *A. dealbata* → *A. mearnsii*	1	3	42.9 (*A. dealbata*)	42.9
			42.9 (*A. mearnsii*)	
Total time on either type of foliage	10	27	38.6	14.3 – 85.7
Day 2				
On no host	13	n/a		
On *A. mearnsii* only	11	47	53.4	12.5 – 100
On *A. dealbata* only	4	17	53.1	12.5 – 100
Moving from *A. mearnsii* → *A. dealbata*	0			
Moving from *A. dealbata* → *A. mearnsii*	3	6	25.0 (*A. dealbata*)	37.5
			41.7 (*A. mearnsii*)	
Total time on either type of foliage	17	83	61.0	12.5 – 75.0

* as a proportion of observational periods. Day 1 = 7 observational periods between 9am and 4 pm, day 2 = 8 observational periods between 8 am and 4pm.

Table 7.11 - Total and average number of eggs laid on *A. mearnsii* and *A. dealbata* in a 48 hour period.

Host plant	Total Eggs	Average ± SE (n)	Range
A. dealbata	55	18.3 ± 11.02 (3)	4 – 40
A. mearnsii	92	23.0 ± 7.43 (4)	2 – 37

Table 7.12 – Breakdown of oviposition behaviour in relation to host visitation.

Number of females	Behaviour	Proportion of females that did lay eggs (%)
2	Laid on *A. dealbata* – did not visit *A. mearnsii*	28.5
3	Laid on *A. mearnsii* – did not visit *A. dealbata*	42.8
1	Visited both *A. mearnsii* and *A. dealbata* – laid on *A. mearnsii*	14.3
1	Visited both *A. mearnsii* and *A. dealbata* – laid on *A. dealbata*	14.3
5	Visited *A. dealbata* and *A. mearnsii* – did not lay	55.6

7.4 Discussion

7.4.1 Infestation of **A. mearnsii** *and* **A. dealbata** *in Tasmania*

During the survey in Chapter 6, field observations suggested that defoliation of *A. mearnsii* commenced only after nearby *A. dealbata* was already mostly defoliated. In this chapter however, it was found that when both species were present in a location they were similarly defoliated, rather than one species being always totally defoliated. The high percentage of both hosts infested at sites combined with the wide distribution of infested *A. dealbata* in Chapter 6 strongly suggests that *A. orphana* oviposits on both hosts when present in an area and not one species preferentially. Further, the greater presence of *A. dealbata* at many of the field sites could lead to the assumption that this species would be the choice for oviposition if the 'patch dynamics hypothesis' (Thompson, 1988) applied to this insect. However, as both species experienced similar defoliation at a variety of sites, then it appears that for *A. orphana*, the more apparent species is not necessarily the primary target for oviposition. Also, since 50% of the infested trees at a site were 10 m or greater apart, it is unlikely that the small larvae of *A.*

orphana would move between trees, further thus supporting the idea that females oviposit on both host species.

Prior to oviposition, females need to select a host based on chemical and visual cues (Beck, 1965; Bach, 1980; Prokopy and Owens 1983; New, 1988; Bernays and Chapman, 1994; Tomlin and Borden, 1996). In another species of paropsine, *C. bimaculata*, Raymond (1998) found *E. regnans* trees with a higher percentage of red foliage experienced significantly more eggs and defoliation. She hypothesized that chemical changes occurring within the trees were one factor affecting the oviposition behaviour of *C. bimaculata*. In this study, *A. mearnsii* foliage was redder in colour than *A. dealbata*. If Raymond's' hypothesis is more widely applicable to other paropsines and host species, then one may expect more eggs to be laid on the redder *A. mearnsii* foliage. However, defoliation scores do not suggest this to be the case in this study.

Nevertheless, the defoliation score in this study can only provide an indication of host choice for oviposition in the field because other factors including; natural enemies, development time on the different hosts and ability of the larvae to feed also affect the defoliation score. Hence, defoliation cannot be directly related to oviposition in the field and all further studies were conducted in controlled conditions.

7.4.2 Larval development on A. dealbata *and* A. mearnsii

The larval-host study identified that overall mortality of *A. mearnsii* fed larvae was comparable with that of larvae fed *A. dealbata* in Chapter 4. The high mortality of *A. dealbata* larvae in this experiment meant a direct comparison was not possible, necessitating the use of the Chapter 4 mortality results. The reason for the high mortality is unknown. It is unlikely to be due to parasitism as firstly, no egg parasitoids are known for this species and secondly, the eggs for both the *A. mearnsii* and *A. dealbata* treatments came from the same group of adults and were randomly allocated to the treatments. So, if parasitism was occurring, it should have affected all treatments. In particular, mortality in the first and second instars was higher for larvae fed *A.*

mearnsii when compared to the Chapter 4 results, but overall mortality was comparable, at 81%. Development times were faster for insects fed *A. mearnsii* during all stages except the first instar, and resulted in total development being 25% faster on this host. Pupal masses were also significantly higher for larvae fed *A. mearnsii*, both suggesting that *A. mearnsii* was a superior food source for *A. orphana*.

The longer development time of larvae feeding on *A. dealbata* means there is an increased risk of mortality due to natural enemies compared to *A. mearnsii* for all stages except the first instar. This phenomenon has been labelled the *slow-growth - high mortality hypothesis* (Feeny, 1976; Benrey and Denno, 1997). Results of Chapter 5 indicate that parasitoids are unlikely to have a large impact on *A. orphana*, as only low levels were identified but the impact of predators has not been examined. The scenario for *A. orphana* development and survival on the two hosts can therefore be outlined as follows;

If larvae hatch and feed on *A. mearnsii*, they develop faster and reach a higher pupal weight which can improve fitness (Blueweiss *et al.*, 1978; Nylin *et al.*, 1996). Faster development may also mean less exposure to natural enemies and higher survival (Benrey and Denno, 1997). If larvae hatch and feed on *A. dealbata*, the development is slower and mortality may therefore be expected to be higher. If however, natural enemies are more prevalent during the first instar, then the slightly faster development time of larvae feeding on *A. dealbata* may be more advantageous than faster overall growth rates.

Roff (1980; 1981) suggests that a trade-off for faster development is a smaller body size. In contrast to this, Nylin and Gotthard (1998) in their review on life-history theory, suggest that many factors affect insect growth rates such that faster developing insects do not necessarily have a reduced body size. One example of fast development as well as a large body size is the Seed beetle, *Stator limbatus* Horn (Fox *et al.*, 1995). The preliminary information obtained here with *A. orphana* supports this hypothesis, as the faster developing larvae on *A. mearnsii* became heavier pupa than those reared on *A. dealbata.* This heavier pupal size may then result in a larger female size and higher fecundity (Honek, 1993).

Host tree moisture content was also examined and was significantly higher in *A. dealbata.* Scriber and Slansky (1981) relate moisture content to leaf toughness and suggest that moisture contents of 70% or greater are optimal for insect survival. However, the 64% moisture identified for *A. dealbata* was less than Scriber and Slansky's (1981) optimum. Furthermore, in contrast to the results for *A. orphana*, Harrell *et al.* (1982) found that larvae of *Chrysomela scripta* Fabricius feeding on leaves high in moisture had a higher pupal mass than larvae feeding on leaves lower in moisture. An alternative hypothesis to explain the lower pupal mass of larvae feeding on the higher moisture content *A. dealbata* may be that larvae need to consume a greater mass of higher moisture foliage to obtain equivalent nutrition.

Nitrogen was lower, but not significantly lower, in *A. dealbata* foliage, supporting the suggestion that larvae may need to consume more *A. dealbata* than *A. mearnsii* to obtain equivalent nutrition. The nitrogen values obtained were also higher than the threshold of 1.7% N, below which Ohmart *et al.* (1987) considered that development would be affected. High nitrogen levels may be expected as *Acacia* species are nitrogen fixing. Unless larvae of *A. orphana* have a higher requirement for nitrogen than other paropsines it is unlikely that nitrogen limited larval development in this study. Although secondary plant substances have been found to have little affect on paropsine larval growth in *Eucalyptus*- (Morrow and Fox, 1980; Ohmart *et al.*, 1985b; Patterson *et al.*, 1996) or *Melaleuca*- feeding species (Maddox, 1995), further investigation would be warranted before dismissing their effect in *Acacia* feeding species.

7.4.3 Oviposition on A. dealbata *and* A. mearnsii

In both experiments (choice and no-choice) females collected from *A. dealbata* did not lay significantly more eggs on *A. mearnsii* compared to *A. dealbata* and vice versa. This indicates that the hypothesis of '*mother doesn't know best*' (Courtney and Kibota, 1990) applies to *A.*

orphana. Furthermore, the lack of significance in the results here suggest that the behaviour is closer to that observed by Berdegue *et al.* (1998) in their study of the Lepidopteran, *Spodoptera exigua* which chose to oviposit on a host which caused slower development, lower survival, larval and pupal masses. Berdegue *et al.* (1998) were unable to offer a full explanation for this contradictory behaviour, but suggested the 'enemy free space' hypothesis. Ohsaki and Sato (1999) make a similar observation in their study of three butterfly species (*Pieris* spp.), where they considered that parasitoids were important in the evolution of host-plant usage. As previously discussed, few parasitoids have been identified for *A. orphana*, but the effect of other natural enemies is not known.

Alternatively, the lack of significant differences between eggs laid on the two different hosts may be an indication of a lack of specialization by *A. orphana*. Janz and Nylin (1997) make the observation that specialist butterflies had a preference for good quality nettles, whilst generalist butterflies did not discriminate between good or poor quality nettles. The trade-off for being a specialist may be less oviposition, due to increased search time whilst a more generalist strategy may result in a greater oviposition period and number of eggs, but on potentially poorer hosts (Nylin and Janz, 1999). The behaviour exhibited by *A. orphana* in this chapter is that of the generalist, whereby eggs are laid on both host types, and females do not appear to discriminate. Observations of *A. orphana* on numerous other species (Table 7.1) further support the suggestion that this insect is a generalist.

Tracking insect behaviour over a two-day period showed that the cage may have been affecting the behaviour of *A. orphana*. The number of insects on foliage increased from 37% on the first day to 57% on the second day, and the average time spent on foliage per insect also increased by 22%. However, the remaining insects did not appear interested in the foliage, and were apparently investing more time into escaping than feeding and ovipositing. This behaviour was identified as '*inhibitory cage environment*' by Withers and Barton Browne (1998). Further evidence supporting the observation that cage effects were inhibiting oviposition behaviour is that in 67% of cages, females only visited one host. In five of the seven cages where eggs were laid, the female did not examine both hosts, but oviposited on the only host she visited. Although no significant host choice was found in the oviposition studies, it was apparent from the observations of oviposition behaviour that beetle activity was inhibited. The usefulness of doing such observational studies in addition to more 'simple' host-choice trials is clearly evident.

To determine more about the oviposition behaviour of *A. orphana* on *A. mearnsii* and *A. dealbata* would require further investigation with larger cages or field studies. Once more definitive results are obtained, repetition of the oviposition and development experiments with *A. orphana* collected from the two different host species on mainland Australia would provide an interesting comparison. Alternatively, repetition using Tasmanian insects and providing them with mainland foliage may provide more insight into the host choice of *A. orphana* as well as being an interesting ecological study into oviposition preference/larval performance.

8. Herbivory of *Acacia dealbata* by *Acacicola orphana*

Abstract

In the field, final instar *Acacicola orphana* larvae often consume foliage and green bark of *A. dealbata*, despite it being a poorer host than *A. mearnsii* (Chapter 7). Consumption and efficiency of conversion of ingested leaves to body mass by final instar larvae were determined to be 0.93 $g^{-1}g^{-1}day^{-1}$ and 39.7% respectively. Bark feeding behaviour was also examined, with results suggesting a diet of both foliage and bark combined is of equivalent nutritional value to a diet of foliage only. In all treatments where larvae were offered a combination of foliage and green bark, bark was consumed, suggesting that green bark is not a lesser-preferred diet than leaves. Evaluation of host nitrogen and moisture showed a two-fold increase in nitrogen levels in November, at the end of the larval feeding season but no significant change in moisture.

8.1 Introduction

Though *A. dealbata* was found to be a poorer larval host than *A. mearnsii* (Chapter 7), it has been observed to regularly experience severe defoliation by *A. orphana.* This defoliation is so considerable that the forestry industry in Tasmania has been discouraged from planting *A. dealbata* as a commercial species (Elliott and de Little, 1985). Severe defoliation of *A. dealbata* may be due to a low ability of *A. orphana* larvae to obtain nutrition from the foliage relative to other insects, thus necessitating consumption of large quantities of plant matter to obtain adequate fuel for ontogeny.

Waldbauer (1968) studied insect food consumption and utilisation in detail and provides formulae to enable determination of the efficiency with which food is converted from plant material into insect body mass (EC) and a consumption index (CI) which relates to the amount of food consumed in a set time period. EC was used by Carne (1966b) who found a value of 18-22% for final instar larvae of *P. atomaria* and S.C. Baker, J.A. Elek and S.G. Candy (unpublished data) who identified ECs of 13 and 24 % for *C. bimaculata* fed with *E. regnans* and *E. nitens* foliage respectively. Efficiency of conversion has been shown to vary with stage, as shown by Carne (1966b) who found EC increased until the third instar, but declined in the final instar. This differs from the results Banno and Yamagami (1989) who studied the cerambycid *Eupromus ruber* (Dalman) finding that EC decreased as larvae progressed through the instar stages. Results for Lepidoptera also vary in the literature, with Satpathi (1993) reporting an increase in EC with age whilst Senapati (1989) found third instar *Heliothis armigera* (Hubner) larvae had the highest EC. Thus, trends in EC appear to be insect specific and may be related to the host quality as observed by Harrell *et al.* (1982) who found larvae fed with more succulent foliage developed faster and had a greater efficiency of conversion than those fed older, mature foliage. They did not however, find a difference in the rate of consumption. Whilst Matsuki and MacLean (1994) do not present EC or CI values for their study on five insects fed with different species whose foliage was harvested at varying dates, they found host quality as measured using leaf toughness, percent nitrogen and moisture varied with harvest date. This variation in host quality at different harvest times was found to affect the relative growth rates of their study insects. In particular, low nitrogen and moisture resulted in a low relative growth rate (Matsuki and MacLean, 1994).

Carne (1966b) did not calculate a consumption index for *P. atomaria* and no other consumption values for Coleoptera in a form that could be readily related to CI were identified from the literature. Waldbauer (1968) cites a number of Lepidopteran examples ranging from 1 – 2.2 and presents information showing that CI increases with stage. Thus, the severe defoliation caused by *A. orphana* larvae could be due to greater consumption of foliage by the larger, later instar larvae. A low consumption index could indicate that poor insect growth may not be due to the nutritional value of the diet. Instead, another factor correlated to foliar intake such as the absence of a phagostimulant (Waldbauer, 1968) or presence of phenolic glycosides (Matsuki and MacLean, 1994) may be responsible. Matsuki and MacLean (1994) identified phenolic glycosides in leaves where the chrysomelids feeding on them, *Calligrapha verrucosa* and *Gonioctena occidentalis* showed a low relative growth rate.

8.1.1 Bark Feeding

During the distribution studies (Chapter 6) it was observed that later instar larvae were present when most defoliation occurred, and these often consumed green bark as well as foliage of *A. dealbata*. Elliott and de Little (1985) also made this observation, and commented also that green bark was chewed following all foliage removal. However, during this study I have observed bark being chewed even when foliage was still present on the trees. Greaves (1966) made similar observations to Elliott and de Little (1985) during a study on *C. bimaculata,* where late instar larvae chewed green bark from *E. regnans*. The hypothesis that late instar larvae feed on bark to evade starvation in areas of dense larval populations is plausible, but it is also possible that bark-feeding behaviour is part of a more complex system. This system may involve larvae either gaining nutritional or other benefits from the bark beyond that obtained from the foliage, such as the improved defense abilities obtained by *Neodiprion sertifer* (Geoffroy) when it feeds on pine bark (Larsson *et al.*, 1986). Alternatively, bark feeding by early instars may change the growth pattern or resource allocation of the host tree, resulting in benefits to individual larvae when in later instars as discussed in Chapter 2 (Section 2.4.3).

The *"resource regulation hypothesis"* by Craig *et al.* (1986) suggests that insect feeding early in a season stimulates nutritious regrowth for insects feeding later in the season. A good example of the resource regulation hypothesis is presented by Wool *et al.* (1999) who found aphids feeding on *Pistacia palaestina* induced changes in the vascular system tissues of the host that increased their ability to access their food supply for a longer period. Whilst *A. orphana* chews green bark, it does not appear to modify the vascular tissues of *A. dealbata*. However, through its feeding behaviour it may stimulate tree 'flush' growth that is high in nitrogen and moisture (Scriber and Slansky, 1981; Landsberg, 1990a,b) which can ultimately benefit insect growth and development (Roininen *et al.*, 1988). Landsberg (1990a) found that *Eucalyptus* dieback trees were more heavily grazed than healthy trees and that the foliage on dieback trees was also nutritionally superior, partly due to the younger age of the re-growth. She also notes the predictability of finding quality foliage may be much higher on trees that have been repeatedly defoliated (Landsberg, 1990a) and hence chronic herbivory of the dieback trees may be a partly self-perpetuating event (Landsberg, 1990b).

Harrell *et al.* (1982) found larvae of the chrysomelid *Chrysomela scripta* Fabricius fed on green shoots when all leaves with 70% moisture content or above were consumed. *Acacia dealbata* was found to have moisture contents below this (see Chapter 7) and thus it is possible that *A. orphana* does not discriminate between green stems or foliage for food. Moderate larval feeding during winter may also promote large amounts of 'flush' growth on *A. dealbata* during the following spring/summer, enabling the new generation of *A. orphana* larvae to obtain foliage high in nitrogen early in their lifecycle.

Thus, in this chapter, the feeding behaviour and consumption of foliage by final instars are examined. The larval choice for plant parts, either green bark or foliage is also investigated and to test if larval feeding enhanced the tree as a resource, the nutrition of trees in the field was also assessed at different time intervals.

8.2 Materials and Methods

Final instar insects were chosen for most studies in this chapter because this stage was present in the field when severe damage was occurring, and field observations were that this stage appeared to frequently consume bark (Chapter 4). Furthermore, fourth instars are heavier than the earlier instars, meaning less error in the measurement of larval weight and foliage consumed. Finally, this stage may also show less discrimination in choice of food material, as their mouthparts are bigger and therefore better developed for chewing tougher plant material than earlier instars.

8.2.1 Consumption by final instar larvae

Fourth instar larvae were collected by removal of infested branches from the field at Arve (43° 09', 146° 50') in the Southern Forests, Tasmania. Groups of 10 randomly selected larvae were placed in each of 36 plastic Petri plates (9 cm dia.), all containing moistened filter paper to reduce desiccation. Larvae were starved for 24 hours to eliminate food in the gut. After this, larvae in each dish were weighed and a known (excess) mass of fresh *A. dealbata* foliage provided. The larvae were kept at 18 ± 2 °C (24L: 0D) in a constant temperature room. Twelve control dishes containing only moistened filter paper and fresh foliage were also incubated at the commencement of the experiment to evaluate foliar moisture loss.

Three treatment dishes and one control dish were removed from the constant temperature room every four hours up to 48 hours. As each time treatment was removed, larvae were separated from the foliage and any deaths noted. The foliage was immediately re-weighed. Larvae were again starved for 24 hours and re-weighed.

Net consumption was graphed against time. The efficiency of conversion of ingested food to body substance (EC) was calculated from equation 1 (Waldbauer, 1968).

$$EC = \frac{\Delta larval\ \ mass\ \ (g)}{\Delta food\ \ mass\ \ (g)} \times 100 \qquad \text{Equation 1}$$

8.2.2 Influence of plant material on insect choice and feeding

8.2.2.1 Within tree larval food choice

One hundred field-collected fourth instar larvae were starved for 24 hours and then randomly placed in cohorts of 20 on the foliage of 5 potted *A. dealbata* trees in a glasshouse (temperature 20 ± 5 °C). Card-board funnels were sealed around the base of each tree allowing larvae that fell off to easily climb back onto the host. After 3 hours the number of larvae feeding on a) bark and b) leaf material were counted. Preliminary trials leaving larvae on foliage for 24 and 12-hour periods provided no results (ie. no larvae remained on trees) thus, the three-hour time period was considered appropriate.

8.2.2.2 Growth of final instar larvae on bark and leaves

Fourth instar larvae were collected from the field and randomly selected groups of 10 were allocated into 12 plastic Petri dishes (9 cm dia.) lined with moistened filter paper. After starving the insects for 24 hours they were weighed and provided with either pinnules from *A. dealbata,* green bark material or a combination of 50 % (by weight) of both. The treatments were replicated four times. The containers were sealed and incubated at 17 °C ± 1 °C; 8L:16D; ~66% R.H for a period of 8 days (approx. 40% of total fourth instar duration). Foliage was replaced at day 4 to ensure foliage freshness. On day 8 larvae were removed from the foliage, starved for 24 hours and re-weighed. The mean larval mass increments were determined. Analysis of variance was conducted in Genstat 5 v3.2.

8.2.2.3 Larval development on different types of plant material

To investigate the effect of plant material on larval survival and development, adults were collected from *A. dealbata* trees at Buckland (42° 39'S, 147° 36'E) in April 1998 and placed in a cage in the laboratory. After 24 hours all eggs were collected from the foliage and groups of 10 eggs were randomly placed in each of 16 plastic Petri plates (9 cm diameter) lined with moistened filter paper. These were incubated in Contherm CAT 150 MCP cooled incubators at 17 °C ± 1 °C; 8L:16D; ~66% R.H.

Upon hatching, larvae were fed a diet of young foliage, intermediate foliage, old foliage or green bark material (see Table 8.1). Abundant fresh food was provided three times weekly from the same tree. There were 4 replicates for each food type.

Stage specific mortality was determined and development time was measured in days on an average 'per dish' basis.

Table 8.1 - Foliage types and descriptions

Foliage type	Description
Young Foliage	- newly formed leaf, taken from the growing tip of a branch
Old Foliage	- collected from the base of a branch, close to the stem of the tree
Intermediate	- intermediate between young and old foliage types and
Green Bark	- removed from branches less than 5 mm in diameter

Nitrogen content of the different foliage types and bark was quantified using the wet-digestion method outlined in Chapter 7 (Lowther, 1980). Percent moisture of the different plant materials was evaluated on a wet weight basis.

8.2.3 Nitrogen and moisture content of A. dealbata *after defoliation*

To determine whether severe feeding by larvae of *A. orphana* results in improved nutritional quality of *A. dealbata* foliage in the following season, the nitrogen and moisture contents of 54 *A. dealbata* trees were assessed. To ensure foliage was available for these nutrient tests in November, when the trees could be severely defoliated, sample branches were identified during June 1997, sprayed with an insecticide to ensure no larvae were present and encased in a fine mesh bag. Early in November 1997, at the end of the larval feeding season, the bags were removed for foliage analysis and the tree scored for defoliation (See Chapter 6 for defoliation scale). The undamaged foliage in the bags was analysed for nitrogen and moisture content in the laboratory using the same method as used in Chapter 7 (Lowther, 1980). Six months following defoliation, on April 6 1998, regrowth foliage was assessed for nitrogen and moisture content.

Whilst it was intended to continue this study for two seasons, removal of branches containing bags (vandalism) and roadwork resulted in a 44% loss of sample trees during the first 6 month period. Only 30 of the original trees were remaining at the April sample date and further reductions during 1998 meant that insufficient sample sizes were available to continue the study beyond the first season.

8.3 Results

8.3.1 Foliage consumption by final instar larvae

Total foliage consumption was low until approximately 12 hours after incubation, possibly due to handling. Thereafter, consumption increased with time (Figure 8.1), although considerable variation is apparent. Each insect consumed an average of 6.99mg (range 3.39 – 17.41mg, median = 7.26mg) of foliage per day (Table 8.2). Average body mass of the insects was 7.53mg. Hence, the average consumption index was estimated to be 0.93 $g^{-1}g^{-1}day^{-1}$. This indicates that larvae consume almost the equivalent of their body weight each day.

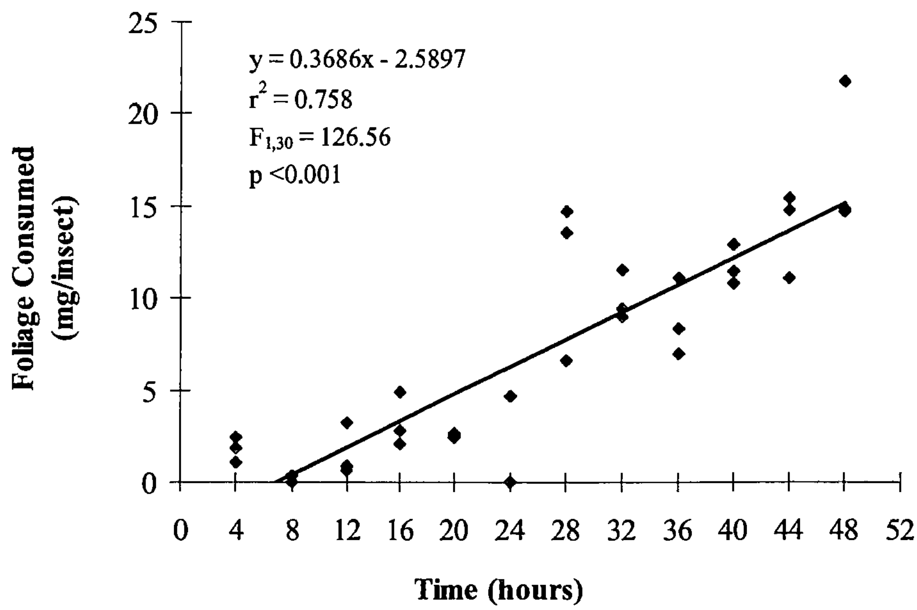


Figure 8.1 - Consumption of *A. dealbata* foliage by fourth instar larvae over 48 hours.

When the consumption index was re-calculated using data post-16 hours into the experiment to account for possible handling stress, the amount consumed was still significantly correlated with body mass, and the resulting CI was only slightly higher at 0.97 $g^{-1}g^{-1}day^{-1}$ (Linear regression: $r^2 = 0.712$, $p < 0.001$). Overall, the efficiency of conversion (EC) of fresh foliage into body mass was calculated to be 39.7%.

Table 8.2 – Average amount consumed and average weight increment of fourth instar larvae fed *A. dealbata**

	Count (n)	Average ± SE (mg)	Minimum (mg)	Maximum (mg)
Amount consumed per insect	25	6.61 ± 4.921	2.97	12.61
Weight increment per insect	26	2.55 ± 0.380	1.46	11.54

*all averages calculated from results for 16 hours onwards.

8.3.2 Influence of plant material on insect choice and feeding

8.3.2.1 Within tree larval food choice

Only 28 of the original 100 larvae were located on the trees after three hours. The location of the remainder of the larvae was not determined. Of the larvae recovered, 15 (54%) were found feeding on the green bark, whilst 13 (46%) were found feeding on leaf material (Table 8.3). A paired t-test within plants showed no significant difference in larval numbers between leaf and bark ($t = 2.77$, $p = 0.717$, $df = 4$). This suggests larvae did not select one food source over the other.

Table 8.3 - Positions of fourth instar larvae on potted trees after 3 hours.

Position	Average ± SE (n)	Range (no. larvae recovered per tree)	Total larvae recovered
Leaves	2.6 ± 0.68 (5)	1-6	13
Bark	3.0 ± 0.95 (5)	1-5	15

8.3.2.2 Growth of final instar larvae on leaves and bark

Larvae fed on the foliage provided in all treatments, with those in the leaf and bark combination consuming both leaf and bark material. Larvae fed leaf or the combination gained significantly more weight gain than those in the green bark treatment alone ($F_{2,6} = 9.10$, $p = 0.015$). There was no significant difference between weight gained by larvae fed leaves only or the combination (Table 8.4). This suggests that foliage is necessary in the diet.

Table 8.4 - Growth increment and least significant difference of fourth instar larvae feeding on leaves, bark or a combination of *A. dealbata* foliage.

Treatment	Average ± $SE^{a\text{-}b}$ Mg (n = 4)	Min. (mg)	Max. (mg)
Leaf	4.13 ± 0.44^a	2.83	4.70
Leaf and green bark	3.43 ± 0.48^a	2.51	4.48
Green bark	2.05 ± 0.67^b	0.17	3.33

$^{a\text{-}b}$LSD = 1.21

8.3.2.3 Larval development on different plant material

Significant differences between treatments were observed for both percent moisture (Repeated measures: $F_{3,6} = 7.54$, $p < 0.05$) and percent nitrogen (Repeated measures: $F_{3,6} = 32.46$, $p < 0.001$) (Table 8.5). Green bark and young foliage both had significantly higher moisture contents than old foliage. The intermediate foliage was equally low in moisture to the old foliage, but not significantly lower than the bark and new foliage (Table 8.5). Nitrogen content was not significantly different between young and intermediate foliage but both were significantly higher in nitrogen than old foliage or green bark material. Green bark was significantly lower in nitrogen than all other foliage types (Table 8.5).

Table 8.5- Moisture content and total nitrogen (± SE) of the different foliage types.

Foliage type	Count	Moisture (%)	Total Nitrogen (%)
Young	3	73.0 ± 4.84^{a}	3.32 ± 0.261^{a}
Intermediate	3	63.2 ± 4.50^{ab}	3.05 ± 0.125^{a}
Old	3	53.1 ± 0.63^{b}	2.56 ± 0.210^{b}
Green Bark	3	67.8 ± 1.09^{a}	1.37 ± 0.157^{c}

$^{a\text{-}c}$ numbers with different letters in the same column are significantly different (Moisture: LSD = 10.64; %Nitrogen: LSD = 0.4677)

Quantification of larval development on the different plant tissues was limited by high mortality across all treatments and replicates. Mortality of each stage and treatment as a proportion of larvae developing to that stage is shown in Table 8.6. Only young and intermediate foliage diets resulted in any larval survival beyond the first instar. Of these remaining larvae, none developed to the fourth instar, one larva developed to the third instar, which was in the intermediate foliage treatment. Three individuals developed to the second instar on the young foliage diet.

Table 8.6 – Survival of *A. orphana* larvae as a percentage of insects in the previous stage when fed different plant materials.

Foliage type	Survival as a percentage of the previous stage (%) (n)				
	Eggs	First Instar	Second Instar	Third Instar	Fourth Instar
Young	100	78	10	0	
	(40)	(31)	(3)	(0)	
Intermediate	100	85	6	50	
	(40)	(34)	(2)	(1)	
Old	100	93	0		
	(40)	(37)	(0)		
Green Bark	100	68	0		
	(40)	(27)	(0)		

Average development time for first instar larvae fed with young foliage was 31 days (n = 1 replicate), slightly longer than the 28 ± 1 days (n = 2 replicates) observed for the same stage fed with intermediate foliage.

8.3.3 Nitrogen and moisture content of A. dealbata *after defoliation*

Immediately after severe defoliation a significant negative relationship existed between the defoliation level and the percentage nitrogen in the foliage ($F_{1,27} = 7.11$, $p = 0.013$) (Figure 8.2). However, six months after the defoliation event, the relationship between previous season defoliation level and nitrogen content was not significant ($F_{1,27} = 2.09$, $p = 0.159$) (Figure 8.2). A decline (average = 4.6 ± 1.86 %N, range 2.7 – 6.8 %N) in foliar nitrogen occurred between the two sample dates. The foliar nitrogen level of 2.6 %N determined in April 1998 was approximately half the 5.7 %N observed in November 1997 (paired t-test: $t = 14.25$, $p < 0.0001$, $df = 28$) (Table 8.7). A significant negative relationship also existed between the %N decline and defoliation score ($R^2 = 0.158$, $n = 28$, $p = 0.032$). At this time the growth of the tree would be starting to slow with the shortening of days and approach of winter.

Table 8.7 - Average percent nitrogen (±SE) of *A. dealbata* foliage immediately following defoliation and 6 months after defoliation.

Sample Time	Count	Average ± SE	Range
November 1997	28	5.7 ± 0.23	3.6 – 9.0
April 1998	28	2.6 ± 0.06	2.0 – 3.3

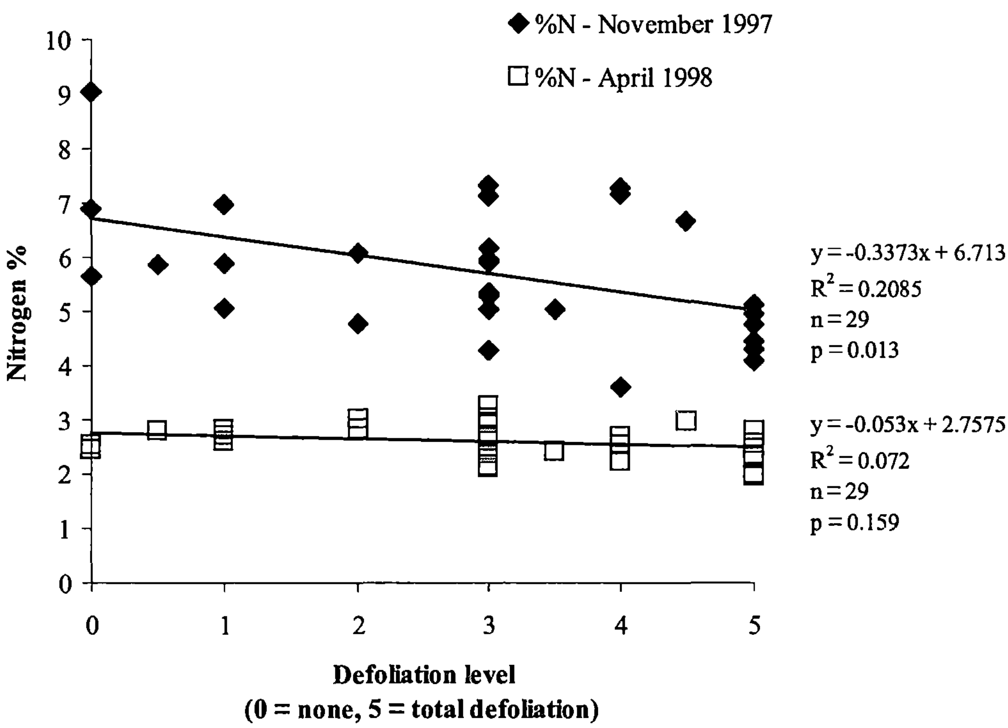


Figure 8.2 - Percentage nitrogen in *A. dealbata* trees with different levels of defoliation immediately after the defoliation event in November 1997 and 6 months following defoliation in April 1998.

No relationship between foliar moisture content and leaf nitrogen was observed in November 1997 ($F_{1,27} = 0.68$, $p = 0.417$; $R^2 = 0.0245$) or April 1998 ($F_{1,27} = 0.69$, $p = 0.412$; $R^2 = 0.0251$).

8.4 Discussion

8.4.1 Foliage consumption by final instar larvae

Fourth instar *A. orphana* larvae consumed approximately the equivalent of their body mass in fresh foliage every 24 hours. The consumption index estimations of 0.93 – 0.97 $g^{-1}g^{-1}day^{-1}$, obtained were slightly lower than the 1.0 – 5.6 $g^{-1}g^{-1}day^{-1}$ identified for a range of Lepidoptera but still comparable (Waldbauer, 1968; Seth and Sehgal, 1989; Mohanty and Mittra, 1992; Satpathi and Ghosh, 1993).

Whilst elimination of gut contents by starving insects before and after experimental investigations reduces the potential for gut contents to affect insect weight, Waldbauer (1968) suggested this practice may be counter-productive. Waldbauer (1968) suggested this practice caused unnecessary stress in insects that graze continuously and may reduce the accuracy of results by restricting consumption. The low consumption observed for the initial 12 hours (25% of the study duration) may have been due to handling or starvation stress, although after this period a steady increase in consumption occurred for the remaining 36 hours of the study.

The consumption index of several Lepidoptera has been found to decrease with larval stage (Chatterfee and Chaudhuri, 1989; Senapati, 1989; Filho and Vendramim, 1989; Satpathi, 1993). Findings of Ohmart *et al.* (1985b) support this, as their observations were that first instar larvae had a high CI, which declined in the later instar stages. It is probable that this would also apply to *A. orphana* and thus the relatively low CI observed may not be indicative of the overall ability of *A. orphana* larvae to feed on *A. dealbata*.

The efficiency of conversion (EC) values for other paropsines were lower than the value of 40% obtained for *A. orphana.* Carne (1966b) estimated ECs 18 – 22 % for fourth instar larvae of *P. atomaria*, and Ohmart *et al.* (1985b) reported 7.1 % reported for the same insect. Both studies used *E. blakelyi* foliage but the difference in EC may be may due the method of EC calculation, as Ohmart *et al.* (1985b) used a dry mass measure, whilst Carne (1966b) utilised fresh weights.

All paropsine values listed are however, comparable to the values of 10.3 - 20.7% observed for non-paropsine, non *Acacia*-feeding insects (Ganga and Nagappan 1983; Satpathi and Ghosh, 1993; Magadum *et al.*, 1996). The higher EC obtained for *A. orphana* may be due in part to the N-fixing capability of *Acacia*. Studies on the *Acacia* seed-feeding bruchid, *Bruchisius sahlbergi* by Ernst (1992) recorded EC values up to 45 %. Mattson (1980) modelled EC levels of several invertebrate herbivores in relation to food nitrogen content and showed an EC of 40% is typical for insects when foliar nitrogen levels are approximately 5%. This prediction agrees with the values obtained in this study for *A. orphana*. For insects such as *C. bimaculata* and *P. atomaria* which feed on eucalypt foliage with nitrogen levels of 0.5 – 2.3 % (Fox and Macauley, 1977; Ohmart *et al.*, 1987) the model predicts an EC of approximately 15% which is also within the range of 7.1 – 24% recorded in the literature.

The CI and EC values obtained for fourth instar *A. orphana* suggest that the extensive defoliation frequently observed in the field when larvae reach this stage is not exaggerated by relative body weight during this stage. Instead, defoliation may result from insect size and numbers alone. In support of this hypothesis, in Chapter 6 a significant relationship was observed between estimates of *A. orphana* number and defoliation.

8.4.2 Influence of plant material on larval choice and feeding

Elliott and de Little (1985) suggested that *A. orphana* only fed on bark and/or stem material when all host foliage was consumed. Complete foliage consumption and hence bark feeding is most likely in the fourth instar of the lifecycle of *A. orphana* since trees would have had already experienced defoliation for 6-7 months without foliage growth, which occurs in spring and summer. However, the results of this study suggest bark feeding is not due to a lack of foliage. Firstly, within three hours of being placed on the foliage of potted trees with no prior defoliation, final instar larvae were found in equal numbers feeding on both green bark and leaf material. Secondly, when provided with a choice of bark and leaf material, larvae fed on both. Nevertheless, fourth instar larvae feeding on bark material alone showed significantly less weight gain than those fed with foliage or a combination of foliage and leaves, indicating bark material alone is a less nutritious diet (Section 8.3.2.2.).

First instar larvae of *A. orphana* can also consume all types of plant material but did not develop beyond the first instar on older leaves or green bark. Both old foliage and bark material were significantly lower in nitrogen than young and intermediate foliage. The old foliage was more brittle than the other foliage types with pinnules breaking easily. Thus, it may have been that first instar larvae feeding on it were not able to consume enough to obtain adequate nutrition. Insufficient consumption for nutrition has been previously shown by Ohmart *et al.* (1987) for larvae of *P. atomaria* feeding on leaves of *E. blakelyi* that were old, tough and low in nitrogen. Ohmart *et al.* (1987) also identified a positive relationship between nitrogen and moisture content of the foliage that was not observed in this study.

That final instar larvae fed bark and foliage did not weigh significantly less than those fed only leaf material, may also indicate that *A. orphana* can be an indiscriminate feeder in later stages. Given such potential, it is possible that final instar *A. orphana* could consume a higher proportion of indigestible fibre in the form of green bark (Scriber and Slansky, 1981) without significantly limiting development. The comparatively high efficiency of conversion of ingested food may also partly compensate for the poorer nutritional value of bark. Another reason for the high levels of damage to trees in the field may be over-exploitation of the food source by early instar larvae, thus forcing later instars to consume sub-optimal foliage or green bark to obtain appropriate levels of nutrients (Waldbauer, 1968; Ohmart *et al.*, 1985b; Raubenheimer, 1992; Patterson *et al.*, 1996). Furthermore, larvae of *A. orphana* develop during winter, when *A. dealbata* development would be slowed due to the cooler temperatures.

Nitrogen contents of the different diets except bark were in the range of approximately 2.5 – 3.3% which is lower than predicted by Mattson (1980) but higher than the threshold of 1.7% determined for optimal growth by Ohmart *et al.* (1987). Although lower, bark material was approximately 1.3 %N, which is still above the 1% threshold below which larvae were found to die (Ohmart *et al.*, 1987).

8.4.3 Insect feeding and its influence on host quality

Nitrogen levels observed in November 1997, immediately following the defoliation were approximately 2-fold greater than in April 1998, by which time the trees had made a recovery and were re-foliated. This finding suggests that defoliation does not enhance the nutritional quality of *A. dealbata* for subsequent generations of *A. orphana*, a result that contrasts with Landsberg (1990a). Rather, it is possible that stress from defoliation results in increased nitrogen levels at the end of the larval feeding period. Nitrogen contents 6 months prior to November were not obtained, however it is unlikely that nitrogen levels at the two sample times would be substantially different on a yearly basis. This is because trees in the study sites had been observed defoliated at the same time for the previous two years. Furthermore, the nitrogen levels recorded in April 1999 in Chapter 7 for trees with no defoliation in the previous three years were comparable to the April 1998 nitrogen levels.

One possible hypothesis to explain why *A. orphana* feeds on bark may relate to defence. Larsson *et al.* (1986) found Scots pines (*Pinus sylvestris*) that were low in terpenes had more bark consumed by the hymenopteran, *Neodiprion sertifer*. Low terpene bark was high in resins, which were used for defence by the sawfly. Thus, *A. orphana* larvae may chew bark to sequester potential defence chemicals that they later emit from glands on T8 of the abdomen (see Chapter 2). Moore (1967) found secretions from the glands of *P. atomaria*, *C. amoena* and *C. variicollis* contained hydrogen cyanide, a substance the beetles manufactured from chemicals in the foliage of their hosts. Personal experience is that the secretions from *A. orphana* have a similar odour to *Acacia* oils. Chemical evaluation of the secretions from *A. orphana* has not been conducted.

9. *Intra-specific differences in* Acacia dealbata *and their relationship to first instar survival and seasonal defoliation by* Acacicola orphana.

Abstract

First instar larval survival and defoliation at the end of larval feeding caused by a natural population of *A orphana* were assessed in four provenances of *A. dealbata* in the Florentine Valley in southern Tasmania. One genotype experienced significantly less natural defoliation and lower survival than the others. This provenance had foliage that was less red and lighter in colour than the others, and also had higher nitrogen contents. Relationships between host characteristics such as foliar colour, nitrogen, moisture, pinnule length and fluctuating asymmetry were examined. Trees experiencing greater defoliation and/or lower insect mortality were found to have longer pinnules, redder foliage, lower nitrogen and lower moisture contents. The relationships between fluctuating asymmetry and defoliation or larval mortality were not significant, but fluctuating asymmetry was greater in the more damaged trees. It is hypothesised that abiotic factors affecting the hosts were more important in the host-plant interaction than genetic differences.

9.1 Introduction

9.1.1 Intraspecific differences between plants and herbivory

Acacicola orphana is a widespread pest of *A. dealbata* and *A. mearnsii* is SE Australia yet earlier observations (Chapter 6) indicated that not all trees at a site experienced severe defoliation. The difference in defoliation between trees may be related to site differences that affect tree nutrition and subsequent larval nutrition (Scriber and Slansky, 1981). Trees high in nitrogen and moisture have been associated with superior larval performance due to the higher nutritive value of the foliage (Scriber and Slansky, 1981; Ohmart *et al.*, 1985 a, b; Ohmart *et al.*, 1987). More moist foliage has also been found to be less tough and easier for larvae to feed upon (Ohmart *et al.*, 1987) which is of particular importance to young larvae that may have small or poorly developed mouthparts for chewing older, more mature foliage.

Larval growth has also been related to tree growth rates, where larvae feeding on faster growing, more vigorous trees show enhanced growth (*"plant vigor hypothesis"* - Price *et al.*, 1990; Price, 1991; Preszler and Price, 1995). Variation in tree growth rates and subsequent larval growth could be due to several factors including tree nutrition or climate.

An alternative hypothesis for the variation in defoliation between trees at a site may include factors that affect oviposition such as colour. Oviposition was discussed in Chapter 7, where it was found that although *A. dealbata* was less red than *A. mearnsii*, it experienced similar levels of defoliation. In contrast, Prokopy and Owens (1983) found oviposition could be affected by plant colour (particularly hue and intensity) at close range. Other studies have related red foliage colour to increased herbivory of *Eucalyptus* (Raymond, 1998) and to climate induced stress (Nozzolillo *et al.*, 1990).

9.1.2 Herbivory and provenance trials in Forestry

Forestry trees are susceptible to a range of insect herbivores and whilst chemical control methods are available, by the time damage is apparent it is usually too late for effective action (Farrow *et al.* 1994). An alternative and more 'environmentally friendly' insect management option may be selection of resistant or less-preferred plantation trees. Farrow *et al.* (1994) investigated the resistance of *E. globulus* provenances to the autumn gum moth (*Mnesampela privata* Guenee) and leaf blister sawfly (*Phylacteophaga froggatti* Riek) finding damage was significantly less in one provenance. Raymond (1995) also examined defoliation of *Eucalyptus nitens* and *E. regnans* in Tasmania in relation to *C. bimaculata* feeding, finding significant and repeatable differences between families. Using the information on susceptibility from Raymond (1995), Patterson *et al.* (1996) evaluated survival and growth of first instar *C. bimaculata* larvae, finding no significant differences between the families. They suggested that whilst herbivore resistance mechanisms in *E. regnans* did not affect larval survival and growth, there may be an effect on adult feeding or oviposition (Patterson *et al.*, 1996). Furthermore, whilst 11 of 24 tree-growth and foliar properties identified were significant at a family level, none appeared to significantly affect larval growth and survival, again supporting the idea that later stage larvae, oviposition and adult feeding behaviour were important in terms of damage to trees (Patterson *et al.*, 1996).

Temperate *Acacia* species provenance trials in Australia are few. Many trials overseas aim to find species with optimum wood production, and are not concerned with provenance level variation or insect infestations (eg. Cossalter, 1986; Logan, 1986; Kessy, 1986; Kaumi, 1986; Fangqiu *et al.*, 1998). In southern Tasmania, an *A. dealbata* provenance trial was established in the Florentine Valley in 1993. The aim was to evaluate differences in plant growth characteristics and insect resistance. This trial has experienced minimal infestation by *A. orphana* and other insects since planting (personal observations) and it has not been possible to draw conclusions relating to provenance or family susceptibilities to *A. orphana*. Hunt *et al.* (1996) examined chrysomelid herbivory of *A. dealbata* and *A. mearnsii* on different provenances in SE Australia, but did not evaluate levels of herbivory on the different provenances.

9.1.3 Stress indicators

Stress affecting organisms can be either environmental or genetic (Palmer and Strobeck, 1986). Genetic characters such as hybridization and inbreeding are not influenced by non-heritable factors, and can therefore be separated from environmental stresses when examining flora and fauna. Environmental stresses include; climate, fertilisers, predators and pathogens (Moller, 1993; Watson and Thornhill, 1994; Moller, 1995). One measure of environmental stress that can be applied to plants and animals is fluctuating asymmetry. This is an indication of stress during organism development (Palmer and Strobeck, 1986; Watson and Thornhill, 1994; Moller, 1995; Tomkins and Simmons, 1995; Sullivan, 1998; Hardersen, 2000; Badyaev *et al.*, 2000). Apart from fluctuating asymmetry there are two other types of asymmetry; directional asymmetry and antisymmetry (Palmer, 1994). Of particular interest to this thesis however, is fluctuating asymmetry (hereafter referred to as FA), as it provides a quantifiable estimate of the amount of variation due to environmental stress during the development of the organism and in the relative form is comparable between and across organisms. Fluctuating asymmetry has an underlying assumption that departures from symmetry are not heritable in an individual and are therefore due to developmental instability caused by the environment (Palmer, 1994).

Palmer (1994) provides the following explanation of FA;

FA is... "*a pattern of bilateral variation in a sample of individuals, where the mean of right minus left is zero and the variation is normally distributed about that mean.*"

Fluctuating asymmetry studies involving insects may have implications for pest control programs as shown by Naugler and Leech (1994) in their study of the forest tent caterpillar, *Malacosoma disstria* Hubner. They showed that FA of the first tarsal segment of the proleg was significantly and inversely related to survival ability in controlled conditions. They hypothesised that FA measures could be appropriate to assess health of a caterpillar population in field conditions and thus the efficacy of chemicals used for control. In another study using the bushcricket, *Sciarasaga quadrata*, Hunt and Allen (1998) found fluctuating asymmetry was related to male bushcricket parasitism, and hypothesised that males with higher fluctuating asymmetry in their hind tibia produced shorter calls, which promoted host location by an acoustically orienting parasitoid.

As well as being related to environmental stress, fluctuating asymmetry has been positively correlated with herbivory by Moller (1995), who identified that trees under stress had a greater number of leaf miners on the foliage than trees that were not. He also subjected trees to environmental stress and found he was able to promote asymmetry and infestation.

9.1.4 Chapter aims

The first aim of this chapter was to evaluate survival and growth of first instar larvae when contained on the foliage of trees of different provenances and families.

The second aim was to examine defoliation caused by a natural population of *A. orphana* in the study area and relate this to *A. dealbata* genotype.

The final aim was to examine phenotypic characteristics of host plants that correlate with low susceptibility to infestation or low survival of *A. orphana.*

9.2 Materials and Methods

Four provenances within a larger pre-established provenance trial were selected based on climatic data generated in BIOCLIM (Table 9.1). BIOCLIM is a bioclimatic database that enables identification of climatic variables including; mean annual temperature, maximum temperature of the warmest period, minimum temperature of the coldest period, annual precipitation, precipitation of the wettest quarter and precipitation of the driest quarter. The four provenances selected were from a hot-dry area (South-east), a cool-wet area (Central highlands) and two locations in between. Within these climatic gradients, sites that were also widely geographically separated were chosen. (Figure 9.1).

Figure 9.1 – Original locations of the four provenances used in the trial and the location of the trial site.

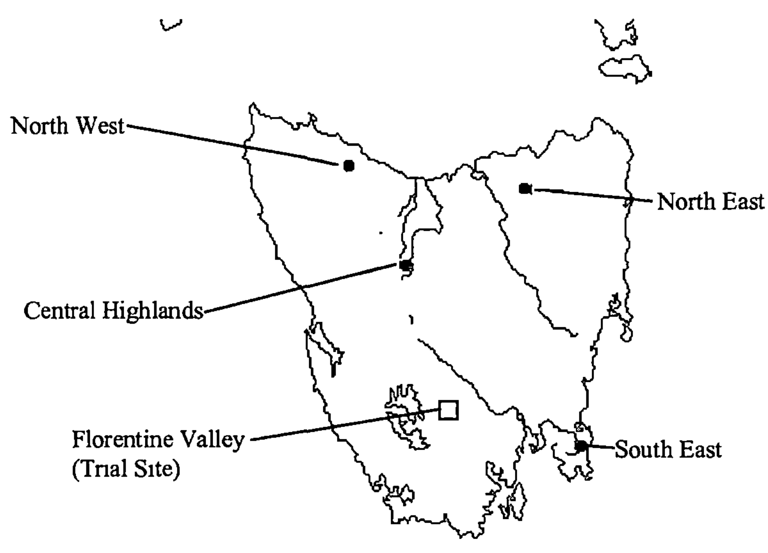


Table 9.1 - Climatic data for the locations from which the four provenances originated and the trial site (Florentine Valley, Southern Tasmania).

	North East (NE)	Central (CH) (cold, wet)	South East (SE) (warm, dry)	North West (NW)	Trial Site (south, central)
Location (lat/long)	41° 15', 147° 25'	41° 46', 146° 10'	43° 00', 147° 55'	41° 05', 145° 37'	42° 21', 146° 28'
Altitude (m ASL)	590	840	110	90	410
Annual mean temperature (°C)	8.3	6.5	11.7	11.7	9.2
Maximum temperature of the warmest period (°C)	17.5	16.2	22	18.2	21.2
Minimum temperature of the coldest period (°C)	2.2	0	2.2	6.7	0.1
Annual precipitation (mm)	2008	2308	581	1078	1404
Precipitation of the wettest quarter (mm)	695	718	159	402	372
Precipitation of the driest quarter (mm)	285	403	129	158	194

The greater provenance trial was established at the Florentine Valley in 1993, and is managed by Forestry Tasmania. The trees were five years old at the time of this study. There were 244 family seedlots from 25 provenances and 3 bulked provenance seedlots planted in an incomplete block design. There were four replicates, each containing 25 incomplete blocks which consisted of 10 plots/incomplete block. Ten trees were planted in each plot.

The plot subset (8% of trees in the greater provenance trial) used for studying *A. orphana* infestation and survival in this chapter are presented in Figure 9.2. To enable assessment of provenance, family and tree effects, a nested design was used. Thus, within each of the four provenances three families were randomly chosen (labels: NW 1-3, CH 4-6, NE 7-9, SE 10-12), giving a total of 12 families. In each family plot three trees were randomly selected, resulting in 108 trees total for the trial.

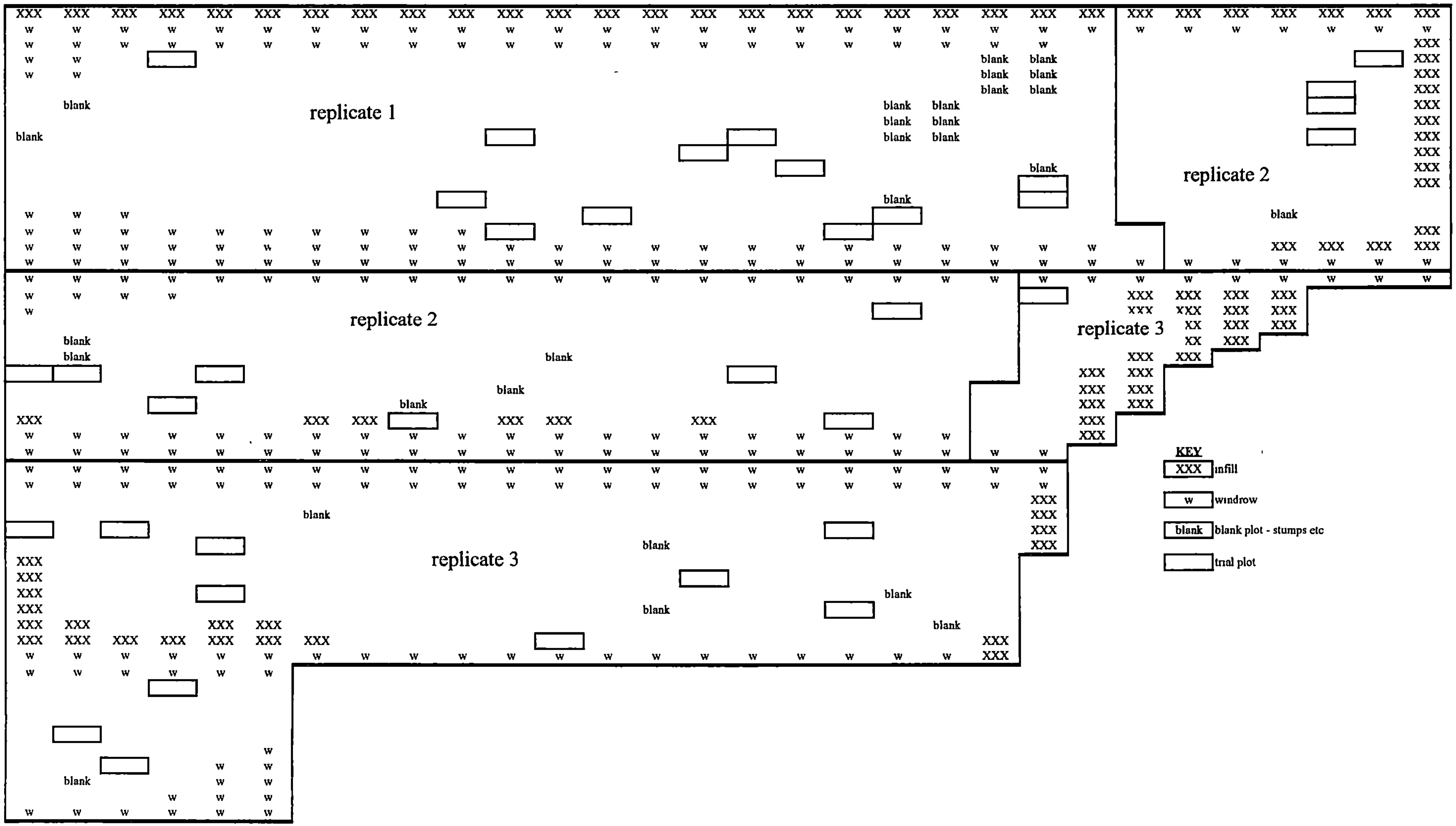


Figure 9.2 - Plot locations

Eggs were collected from caged field-collected adults (from Arve, in the south east of Tasmania – see Chapter 4) which were fed with *A. dealbata* foliage in the laboratory. Eggs were collected over a one week period and were incubated or refrigerated as necessary to ensure that development was as even as possible. The developing larva could be seen through the chorion (see figure 3.2, Chapter 3) enabling checks on the development of larvae within the eggs. At the end of this period, the leaflets containing eggs were randomly sorted into groups of approximately 20 eggs (range 18-23) and tied together with cotton.

On April 20th 1998 one group of approximately 20 eggs was tied to each of three terminal leaves on each of the 108 trees with cotton. Branches for placement of the eggs were selected at random. Approximately 30 cm of each branch tip containing the egg group was then enclosed in a gauze cloth mesh bag measuring 40 cm wide and 50 cm long. The bag was securely tied to the branch with elastic so hatched larvae could not crawl out. Before putting eggs on the leaves, the foliage was checked for eggs (laid by *A. orphana* already present at the site) and predators and, if any were found they were removed. First instar larvae were selected for this study as these would have few reserves and be more sensitive to differences in foliage, resulting in a greater impact on feeding ability and survival than the older instar larvae used in Chapter 8.

After 6 weeks in the field, it was estimated (from Chapter 4 development information) that larvae would be nearing the completion of their first instar stage. Some bags were then untied and larvae checked for development. At this stage many larvae were small, and showed no indication of nearing their moult to the second instar. Hence, it was decided to leave larvae on the trees for a further 2 weeks. After this time the branch tips enclosed in bags were cut from the trees and taken to the laboratory where they were frozen immediately at –20 °C. Following this, bags were cut from the branch tips when measurements were to be taken, and the larvae removed for counting and measurement. Foliage was processed to enable measurement of tree characters.

9.2.1.1 Tree characteristics

Moisture

The branch tips were removed from the gauze cloth bags and larvae collected. The foliage was then weighed, oven dried at 80 °C for three days and re-weighed to enable determination of moisture content.

Nitrogen

The oven dried foliage was ground in a hammer mill and a sub-sample analysed for total nitrogen using the wet digestion method outlined in Chapter 7, section 7.2.2 (Lowther, 1980).

Colour

Colour of a sub-sample of the ground foliage from each branch was assessed in terms of lightness (L*), a* and b* (chromaticity indices) using a Minolta Chromo Meter CR200-b (Minolta, Japan) as described in Chapter 7, section 7.2.2.

Tree height and diameter at breast height (DBH)

Tree height and diameter at breast height (DBH) were measured in May 1998 and again in April 1999 to enable comparison of tree growth increments. Tree volume was calculated using equation 1. Trunk volume was calculated based on the model that a tree trunk is cylindrical up to 1.3 m high and conical from 1.3 m upwards (Patterson *et al.*, 1996).

$$\text{Trunk volume} = \frac{\left[\pi\, r^2 (h-1.3)\right]}{3} + 1.3\pi\, r^2 \qquad \text{Equation 1}$$

Where r = tree radius and h = tree height.

Growth rate of the trees was calculated by comparing differences in tree heights, DBH's and trunk volumes using equation 2 (Patterson *et al.*, 1996).

$$\text{Growth rate} = \frac{\left(\ln T_{\text{April 1999}} - \ln T_{\text{May 1998}}\right)}{t} \qquad \text{Equation 2}$$

Where T = tree height, DBH or volume and t = time (11 months).

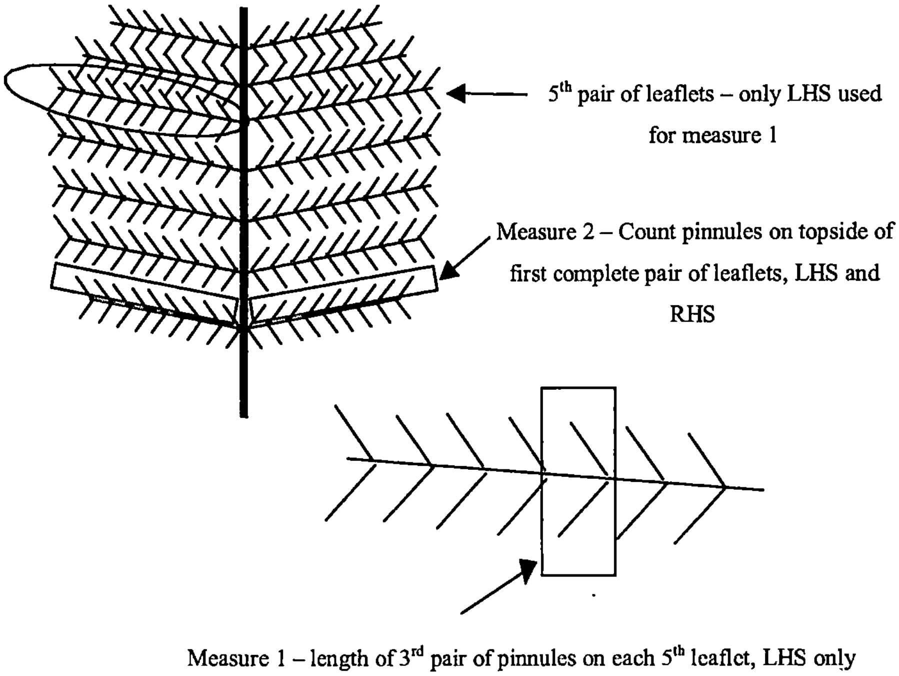
Fluctuating asymmetry

Before oven drying the foliage, the third expanded leaf from the tip of each branch was removed and placed in a separate container for use in the leaf symmetry analysis. If the third leaf was too damaged, then the next closest undamaged leaf was used and so on.

Two different aspects of the leaf were measured to determine symmetry. Multiple measures of symmetry are considered desirable for such studies. One example is Moller (1995) who used multiple traits of elm leaves in his study of leaf miners and FA, to reduce the chance of differences in leaf morphology being a consequence of the insects affecting leaf development. Thus, multiple traits add additional power to the analysis and can enhance the confidence that the pattern observed is 'real' and not just attributable to that particular trait (Palmer, 1994).

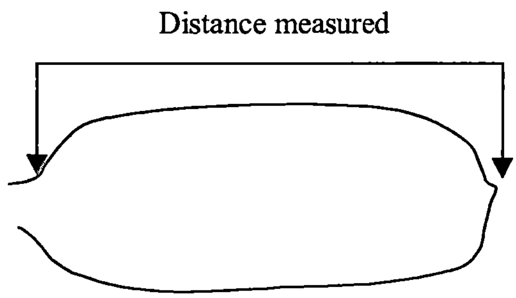
The first leaf parameter measured was pinnule symmetry. For pinnule symmetry, the 3rd pair of opposing pinnules on the LHS of the 5th pair of leaflets from the base of the leaf (Figure 9.3) were removed and mounted in glycerine jelly (Appendix 5).

Figure 9.3 - Diagram of different parts of leaf measured to determine leaf symmetry.



The pinnules had distinct tip and base structures (Figure 9.4), and the distance between these was measured using a binocular dissecting microscope with a calibrated graticule (divisions = 0.15 mm).

Figure 9.4 - Pinnule measurement points for symmetry measurements



The second measure was pinnule number symmetry. This was measured by counting the number of pinnules from the mid-rib of the leaf to the tip on the upper side of the first complete pair of leaflets (Figure 9.3).

Of the symmetry measurements, it was not possible to accurately count the numbers of pinnules on each side of the midrib for measure 2, due to >50% of leaves having missing and/or damaged leaflets. This made it impossible to accurately assess pinnule number. For measure 1, 8 % of pinnules were damaged and hence the analysis contains 25 missing values (25 of 324 leaves were damaged).

All measurements were conducted twice. The time interval between the first and second measurement was longer than the time for making the measurements. The same person made all measurements. A minimum of two measurement replications is required to distinguish true symmetry from measurement error (Swaddle *et al.*, 1994) because of the small magnitude of FA in relation to measurement error. Repeatability of the pinnule length measurements for relative FA was 99.6% ($t = 1.97$, $df = 594$, $p = 0.971$).

A mixed model ANOVA using sides (L or R), individuals and replicate (the repeated measures) entered as main factors provides an interaction variance, individual-by-sides, that is an estimate of the asymmetry variance (Palmer, 1994; Hunt and Simmons, 1998). The resulting F-test indicates whether between-individual variation in estimated asymmetry is significantly greater than that accounted for by measurement error (Swaddle *et al.*, 1994). This test showed that asymmetry was significant ($F_{107,216} = 3.730$, $p < 0.0001$) and that measurement error was 4.53%.

The next step required determining if the signed asymmetries were normally distributed around a mean of zero. A histogram examining the distribution of the signed absolute FA showed it was not normally distributed about zero, and this was confirmed using a one sample t-test ($t = 12.05$, $df = 298$, $p < 0.001$, mean = 0.2098, variance = 0.089). A test for directional asymmetry at this stage showed that this was present, as the right side was significantly longer than the left ($t = 1.97$, $df = 298$, $p = 7.1 \times 10^{-28}$). Examination of directional asymmetry values on a provenance and family level showed no significant differences (Provenance: $F_{3,191} = 0.25$, $p = 0.858$; Family: $F_{8,191} = 0.35$, $p = 0.944$), suggesting that directional asymmetry is assigned to the population as a whole.

The values were then transformed by adding half the average (L+R) to the shorter side and subtracting the same from the longer side (Palmer, 1994). The distribution of the data was still significantly different from a normal distribution, and upon closer examination two values (out of 299) were responsible for the deviation. These were eliminated from the analysis, making the distribution not significantly different to the normal distribution ($n = 297$, $p = 0.10$, Filliben, 1975). The relationship between average pinnule length and the absolute corrected FA measures was significant ($F_{1,106} = 9.62$, $p = 0.002$) and thus the data was converted to relative FA by dividing the absolute FA values by the average pinnule length. This enables comparisons of the size of the asymmetry in relation to the trait (Swaddle *et al.*, 1994). The regression of relative FA and pinnule length was not significant ($F_{1,106} = 0.608$, $p = 0.437$).

Significant differences in relative and absolute fluctuating asymmetry between trees, families and provenances were determined using a general linear model analysis in SAS (SAS Institute, 1989).

9.2.1.2 Insect variables

Two independent measures of tree susceptibility to Fireblight beetle were undertaken. These were performance of first instar larvae and 'natural defoliation'. The term 'natural defoliation' refers to defoliation caused by a natural population of *A. orphana* that was present in the study area. This defoliation was assessed at the end of the 1998 larval feeding period, when larvae were entering pupation.

First instar survival and growth

Three measures of larval performance were measured; recovery, larval wet weight and larval dry weight. Recovery of larvae from each branch was calculated as a percentage of the number of eggs originally placed on the foliage and is hereafter referred to as larval survival. Larvae collected from each branch were then weighed as a group on a microbalance (units = µg), oven dried at 80 °C for three days and re-weighed to obtain group dry weights.

Defoliation by a natural fireblight beetle population

The level of defoliation experienced due to a naturally occurring population of *A. orphana* was assessed in October 1998, using the 5-point defoliation level system outlined in Chapter 6, Section 6.2.1. These trees were the same trees that the larval performance was assessed upon.

9.2.2 Analyses

A general linear model in SAS (SAS Institute, 1989) was used to determine significant differences at the provenance, family and tree level for each of the variables outlined in the preceding paragraphs. To fulfil the assumption of normality, the values obtained for survival were log transformed.

Significant correlations between the variables were identified by construction of a correlation chart involving all tree and insect characteristics. Once the significant correlations were determined, further information including a p-value for each significant interaction was evaluated using a Spearman Rank test (Genstat 5 v3.2).

Relationships between the different variables assessed on a tree level were analysed using multivariate analysis (in the form of ordination) in PATN, a pattern analysis program (L. Belbin, CSIRO, 1995). The main aim of ordination is to represent sample and species relationships as accurately as possible in a two-dimensional graph (Gauche, 1982). In this study, trees which are similar are near each other on the two-dimensional graph whilst those that are dissimilar are far apart.

Stepwise multiple linear regression used to determine the variables that most affected natural defoliation and insect survival. The stepwise regression initially consisted of all possible variables, and these were eliminated in a stepwise manner, allowing determination of the main interactions.

9.3 Results

The emphasis of this chapter is on provenances, but significant family differences have also been covered in some detail. Whilst many tree characteristics and insect variables analysed in the general linear model were significant at the tree level (Table 9.2), no individual tree information is provided due to the complicated means tables generated from the analysis.

Table 9.2 – Significance (p) of the different variables measured at Provenance, Family and Tree level.

Variable	Provenance	Family	Tree
Tree Nutrition			
Nitrogen	0.007	ns	0.0001
Moisture	ns	ns	0.0018
Colour			
ΔE	ns	ns	0.0001
L*	0.029	ns	0.0001
a*	0.020	ns	0.0001
b*	ns	0.01	0.0007
Insect variables			
Natural defoliation %	0.005	0.007	NR
Survival	ns	ns	0.017
Larval fresh weight	ns	ns	ns
Larval dry weight	ns	ns	ns
Tree growth variables			
Height increment	ns	ns	NR
DBH increment	ns	0.0004	NR
Volume increment	ns	0.013	NR
Growth rate - Height	ns	ns	NR
Growth rate - DBH	ns	0.0001	NR
Growth rate – Volume	ns	0.0001	NR
Symmetry variables			
Average pinnule length	ns	ns	0.0001
Relative FA	ns	ns	ns

NR = no results at tree level due to insufficient levels in the analysis; ns = not significant.

9.3.1 Tree characteristics

The statistical significance of each of the different tree characteristics at the provenance, family and tree level (as determined from the general linear model analysis) are shown in Table 9.2.

The tree growth variables are all inter-related, and are only evaluated on a provenance or family level. The DBH and height measures are used to determine volume and thus individual measures for height and DBH are not discussed in detail.

Whilst no significant differences were observed for the tree measures at the provenance level, the south-east provenance showed the greatest height, DBH and volume increments (Table 9.3a). On average, the north-eastern provenance had the poorest growth increments and slowest rates of the four provenances. Six trees experienced no volume increment, due to tips breaking off or breakage of the main leader.

Significant differences were observed at the family level for four of the six tree measures; DBH increment, volume increment, DBH growth rate and volume growth rate (Table 9.3b). As the DBH measure is a component of the volume equation, for simplification and ease of comparison to other forestry studies (eg. Patterson *et al.*, 1996) the following results will address only volume increments (m^3) and growth rates (m^3yr^{-1}). The family with the greatest volume increment during the trial was number 10, from the SE region of Tasmania. The change in the volume of the trees in this family was 0.16 m^3 and the average increase in DBH was 2.08cm. The lowest volume increments were recorded for the trees in family 5 from the central highland provenance which recorded only 0.028 m^3. The average volume increment was approximately 0.070 m^3.

The volume growth rate was greatest in Family 6, from the CH provenance, at 0.065 m^3yr^{-1}, whilst the lowest was recorded from CH family 5, at only 0.015 m^3yr^{-1} (Table 9.3b).

Overall colour difference (ΔE) between trees was significant at the tree level only (Provenance: $F_{3,216} = 2.86$, $p = 0.104$; Family: $F_{8,216} = 0.92$, $p = 0.505$; Tree: $F_{94,216} = 2.33$, $p = 0.0001$). When divided into its components, both L* and a* were significant at the provenance level (L*: $F_{3,216} = 5.10$, $p = 0.029$; a*: $F_{3,216} = 5.97$, $p = 0.020$; Table 9.4a), indicating that the central highland provenance had significantly lighter and less red (greener) foliage than the other three provenances. Brown colour (b*) was significant only at the family level ($F_{8,216} = 2.72$, $p = 0.010$) (Table 9.2, Table 9.4b). In particular, family 5 of the CH provenance scored the highest brown colour (21.8) whilst family 7 of the north-east provenance scored the lowest (18.8) (Table 9.4b).

Foliar moisture contents were significant at the tree level only and averaged 52.1 % ($F_{94,215} = 1.65$, $p = 0.002$; range: 25.8 – 73.0 %) (Table 9.2). Whilst not significant, the CH provenance had the highest moisture content at 40.4 %, only 3% more than the SE provenance, which had the lowest (Table 9.4a).

Nitrogen content was significant at the tree level ($F_{94,215} = 3.09$, $p = 0.0001$; range 1.72 –3.35 % N) and also at the provenance level ($F_{3,215} = 8.72$, $p = 0.007$) (Table 9.2) where the CH provenance was significantly higher (2.65 %) than the other three provenances (2.32 – 2.38%) (Table 9.4a).

No significant differences were observed for the relative FA measurements in provenances ($F_{3,194} = 1.31$, $p = 0.337$), families ($F_{8,194} = 0.44$, $p = 0.896$) or trees ($F_{94,194} = 0.99$, $p = 0.505$) (Table 9.2; Table 9.5). There were significant differences between trees in the size of the pinnules, which ranged from 1.77 mm to 6.48 mm (average 3.70 ± 0.059 mm; $F_{94,194} = 2.08$, $p = 0.0001$). The stepwise multiple regression showed relative FA was significantly negatively related to moisture content, and positively to volume increment ($F_{2,101} = 3.19$, $p = 0.045$) (Appendix 6).

Table 9.3a - Tree characteristics - growth variables. Mean, standard error and range for all significant variables at the provenance level.

Level	Growth Mean ± SE (range)					
Provenance	Height increment m Not significant	DBH increment m Not significant	Volume increment m^3 Not significant	Growth rate – Height $m. yr^{-1}$ Not significant	Growth rate – DBH $cm. yr^{-1}$ Not significant	Growth rate – Volume $m^3. yr^{-1}$ Not significant
CH	0.60 ± 0.12 (0.00 – 2.03)	1.13 ± 0.14 (0.00 – 2.70)	0.058 ± 0.010 (0.00 – 0.196)	0.087 ± 0.017 (0.00 – 0.305)	0.156 ± 0.023 (0.00 – 0.531)	0.0039 ± 0.0052 (0.0016 – 0.105)
SE	1.20 ± 0.13 (0.00 – 2.54)	1.39 ± 0.16 (0.00 – 2.89)	0.108 ± 0.154 (0.00 – 0.370)	0.162 ± 0.018 (0.00 – 0.319)	0.139 ± 0.015 (0.00 – 0.239)	0.0420 ± 0.0030 (0.0009 – 0.0657)
NW	0.86 ± 0.12 (0.00 – 2.27)	1.38 ± 0.16 (0.00 – 2.80)	0.080 ± 0.118 (0.019– 0.214)	0.130 ± 0.016 (0.00 – 0.280)	0.165 ± 0.018 (0.00 – 0.350)	0.0423 ± 0.0044 (0.0022 – 0.0851)
NE	0.52 ± 0.15 (0.00 – 2.56)	0.65 ± 0.14 (0.00 – 2.80)	0.037 ± 0.088 (0.00 – 0.159)	0.072 ± 0.023 (0.00 – 0.349)	0.083 ± 0.017 (0.00 – 0.383)	0.0264 ± 0.0459 (0.0003 – 0.0790)

Table 9.3b - Tree characteristics - growth variables. Mean, standard error and range for all significant variables at the family level.

Level		Mean ± SE (range)					
Provenance	Family	Height increment m	DBH increment cm	Volume increment m^3	Growth rate – Height $m. yr^{-1}$	Growth rate – DBH $cm. yr^{-1}$	Growth rate – Volume $m^3. yr^{-1}$
		Not significant	LSD = 0.665	LSD = 0.0551	Not significant	LSD = 0.124	LSD = 0.197
SE	10	1.32 ± 0.20	2.08 ± 0.19^{a}	0.159 ± 0.318^{a}	0.170 ± 0.025	0.204 ± 0.011^{ab}	0.0535 ± 0.0026^{ab}
		(0.00 – 2.00)	(1.16 – 2.89)	(0.071 – 0.370)	(0.00 – 0.245)	(0.139 – 0.239)	(0.0429 – 0.0657)
NW	1	0.90 ± 0.22	1.76 ± 0.30^{ab}	0.109 ± 0.022^{ab}	0.135 ± 0.026	0.190 ± 0.031^{bc}	0.0476 ± 0.0064^{ab}
		(0.00 – 2.27)	(0.00 – 2.80)	(0.002– 0.215)	(0.00 – 0.280)	(0.00 – 0.312)	(0.0083 – 0.0747)
CH	6	0.86 ± 0.20	1.72 ± 0.16^{ab}	0.068 ± 0.014^{bcd}	0.144 ± 0.033	0.277 ± 0.036^{a}	0.0654 ± 0.0066^{a}
		(0.00 – 1.77)	(1.09 – 2.70)	(0.012 – 0.016)	(0.00 – 0.305)	(0.153 – 0.531)	(0.0392 – 0.1047)
NW	2	1.11 ± 0.20	1.35 ± 0.24^{bc}	0.087 ± 0.019^{bc}	0.160 ± 0.026	0.150 ± 0.021^{bcde}	0.0416 ± 0.0061^{bc}
		(0.00 – 1.96)	(0.20 – 2.50)	(0.003 – 0.186)	(0.00 – 0.245)	(0.031 – 0.240)	(0.0052 – 0.0628)
CH	4	0.92 ± 0.23	1.18 ± 0.16^{bcd}	0.078 ± 0.020^{bcd}	0.123 ± 0.028	0.134 ± 0.013^{bcdef}	0.0359 ± 0.0043^{bcd}
		(0.00 – 2.03)	(0.62 – 1.90)	(0.027 – 0.196)	(0.00 – 0.246)	(0.089 – 0.199)	(0.0215 – 0.0584)
SE	12	1.45 ± 0.21	1.13 ± 0.16^{bcde}	0.087 ± 0.013^{bc}	0.213 ± 0.024	0.121 ± 0.016^{cdef}	0.0394 ± 0.0044^{bcd}
		(0.67 – 2.54)	(0.24 – 1.80)	(0.035 – 0.158)	(0.094 – 0.319)	(0.027 – 0.186)	(0.0177 – 0.0588)
NW	3	0.56 ± 0.16	1.03 ± 0.25^{cde}	0.042 ± 0.012^{cd}	0.094 ± 0.029	0.157 ± 0.042^{bcd}	0.0379 ± 0.0103^{bcd}
		(0.00 – 1.09)	(0.08 – 1.91)	(0.002 – 0.102)	(0.00 – 0.235)	(0.011 – 0.350)	(0.0022 – 0.0851)
SE	11	0.83 ± 0.26	0.95 ± 0.32^{cde}	0.078 ± 0.025^{bcd}	0.105 ± 0.037	0.091 ± 0.029^{def}	0.0319 ± 0.0084^{cde}
		(0.00 – 1.94)	(0.00 – 2.45)	(0.00 – 0.179)	(0.00 – 0.270)	(0.00 – 0.232)	(0.0009 – 0.0589)
NE	8	0.25 ± 0.16	0.73 ± 0.28^{cde}	0.029 ± 0.011^{d}	0.037 ± 0.027	0.100 ± 0.039^{def}	0.0296 ± 0.0083^{cde}
		(0.00 – 1.08)	(0.00 – 2.80)	(0.00 – 0.085)	(0.00 – 0.176)	(0.00 – 0.383)	(0.0041 – 0.0707)
NE	7	0.62 ± 0.28	0.64 ± 0.28^{de}	0.045 ± 0.020^{cd}	0.094 ± 0.045	0.075 ± 0.031^{ef}	0.0245 ± 0.0102^{de}
		(0.00 – 2.21)	(0.00 – 2.32)	(0.00 – 0.159)	(0.00 – 0.349)	(0.00 – 0.284)	(0.0003 – 0.0790)
NE	9	0.69 ± 0.30	0.57 ± 0.14^{de}	0.038 ± 0.015^{cd}	0.084 ± 0.046	0.076 ± 0.018^{ef}	0.0254 ± 0.0057^{de}
		(0.00 – 2.56)	(0.00 – 1.37)	(0.00 – 0.146)	(0.00 – 0.289)	(0.00 – 0.145)	(0.0020 – 0.0502)
CH	5	0.030 ± 0.18	0.49 ± 0.21^{e}	0.028 ± 0.017^{d}	0.006 ± 0.023	0.057 ± 0.021^{f}	0.0147 ± 0.0063^{e}
		(0.00 – 1.58)	(0.00 – 2.13)	(0.00 – 0.159)	(0.00 – 0.208)	(0.00 – 0.209)	(0.0016 – 0.0574)

Table 9.4a - Tree characteristics – nutrition and colour variables. Mean, standard error and range for all significant variables at the provenance level.

Level	Nutrition Mean ± SE (range)		Colour Mean ± SE (range)			
Provenance	Nitrogen LSD = 0.1713	Moisture Not significant	ΔE Not significant	L* - lightness (LSD = 1.1887)	a* - red LSD = 0.9541	b* - brown Not significant
CH	2.65 ± 0.036^{a}	40.36 ± 0.848	55.71 ± 0.407b	45.0 ± 0.48^{a}	-1.30 ± 0.213^{a}	20.18 ± 0.35
	(1.94 – 3.57)	(13.60 – 50.33)	(46.00 – 62.36)	(37.5 – 57.8)	(-5.9 – 2.3)	(2.2 – 27.1)
SE	2.33 ± 0.041^{b}	37.71 ± 0.910	56.87 ± 0.316ab	43.6 ± 0.38^{b}	-0.11 ± 0.241^{b}	19.80 ± 0.26
	(1.38 – 3.32)	(13.32 – 55.31)	(49.14 – 63.57)	(35.7 – 53.5)	(-4.8 – 5.0)	(14.4 – 25.2)
NW	2.32 ± 0.037^{b}	38.07 ± 0.908	57.09 ± 0.322a	43.5 ± 0.38^{b}	0.04 ± 0.207^{b}	20.28 ± 0.31
	(1.66 – 3.04)	(12.98 – 50.76)	(50.84 – 64.51)	(34.0 – 49.9)	(-5.5 – 5.0)	(12.3 – 25.3)
NE	2.38 ± 0.037^{b}	39.63 ± 0.757	57.30 ± 0.410a	43.1 ± 0.47^{b}	0.32 ± 0.231^{b}	19.59 ± 0.28
	(1.54 – 2.99)	(18.17 – 49.95)	(48.87 – 64.21)	(34.5 – 53.1)	(-5.4 – 5.2)	(13.6 – 23.9)

Table 9.4b - Tree characteristics – nutrition and colour variables. Mean, standard error and range for all significant variables at the family level.

Level		Nutrition Mean ± SE (range)		Colour Mean ± SE (range)			
Provenance	Family	Nitrogen Not significant	Moisture Not significant	ΔE Not significant	L* - lightness Not significant	a* - red Not significant	b* - brown LSD = 1.674
CH	5	2.56 ± 0.050 (2.11 – 3.06)	41.12 ± 1.25 (27.68 – 50.33)	56.33 ± 0.808 (46.00 – 62.36)	44.88 ± 1.02 (37.5 – 57.8)	-0.844 ± 0.39 (-5.9 – 2.0)	21.8 ± 0.47a (17.9 – 27.1)
NW	1	2.36 ± 0.062 (1.73 – 2.98)	39.57 ± 1.39 (27.75 – 50.76)	57.22 ± 0.430 (53.50 – 61.39)	43.56 ± 0.55 (37.8 – 48.4)	0.385 ± 0.35 (-2.8 – 5.0)	21.0 ± 0.45ab (16.2 – 24.6)
NW	3	2.31 ± 0.063 (1.83 – 2.95)	37.98 ± 1.88 (12.98 – 50.34)	57.38 ± 0.551 (52.06 – 63.44)	43.36 ± 0.64 (35.5 – 49.2)	-0.159 ± 0.37 (-3.7 – 4.1)	20.9 ± 0.53ab (14.5 – 25.3)
NE	9	2.33 ± 0.056 (1.86 – 2.92)	37.53 ± 1.47 (18.17 – 47.11)	58.12 ± 0.632 (50.32 – 64.21)	42.34 ± 0.76 (34.7 – 50.9)	0.522 ± 0.30 (-3.0 – 3.0)	20.1 ± 0.48^{bc} (15.1 – 23.9)
NE	8	2.28 ± 0.068 (1.54 – 2.91)	42.06 ± 1.18 (28.71 – 49.95)	57.22 ± 0.803 (49.54 – 64.19)	43.27 ± 0.94 (34.5 – 52.4)	0.796 ± 0.45 (-4.9 – 5.2)	19.9 ± 0.52^{bc} (13.6 – 23.9)
SE	10	2.30 ± 0.066 (1.85 – 3.32)	33.99 ± 1.59 (13.32 – 51.56)	57.97 ± 0.425 (54.72 – 63.57)	42.43 ± 0.58 (35.7 – 47.8)	0.296 ± 0.37 (-2.4 – 5.0)	19.8 ± 0.57^{bc} (15.7 – 25.2)
SE	12	2.33 ± 0.052 (1.71 – 2.85)	41.50 ± 1.54 (27.61 – 55.32)	56.22 – 0.632 (49.14 – 62.90)	44.23 ± 0.756 (35.9 – 53.5)	-0.767 ± 0.39 (-4.8 – 3.3)	19.8 ± 0.39 ^{bc} (14.4 – 23.8)
SE	11	2.35 ± 0.090 (1.38 – 3.16)	38.09 ± 1.24 (27.00 – 48.23)	56.42 ± 0.523 (51.17 – 61.23)	44.01 ± 0.60 (38.5 – 49.5)	0.152 ± 0.47 (-4.4 – 3.7)	19.8 ± 0.38^{bc} (16.4 – 23.6)
CH	4	2.77 ± 0.066 (2.24 – 3.57)	39.84 ± 1.48 (13.61 – 49.31)	55.65 ± 0.668 (48.92 – 60.74)	44.80 ± 0.78 (39.3 – 54.4)	-1.389 ± 0.32 (-4.2 – 1.7)	19.4 0.78^{bc} (2.2 – 25.3)
CH	6	2.60 ± 0.062 (1.93 – 3.33)	40.18 ± 1.67 (18.93 – 50.01)	55.18 ± 0.634 (46.88 – 59.30)	45.17 ± 0.70 (41.4 – 55.1)	-1.670 ± 0.39 (-4.8 – 2.3)	19.3 ± 0.39^{bc} (15.3 – 23.7)
NW	2	2.28 ± 0.068 (1.66 – 3.04)	36.65 ± 1.34 (21.22 – 47.87)	56.66 ± 0.679 (50.84 – 64.51)	43.55 ± 0.81 (34.0 – 49.9)	-0.1074 ± 0.36 (-5.5 – 3.9)	19.0 ± 0.55^{c} (12.3 – 24.7)
NE	7	2.53 ± 0.057 (2.00 – 2.99)	39.31 ± 1.15 (27.93 – 47.97)	56.56 ± 0.678 (48.87 – 63.00)	43.56 ± 0.77 (36.1 – 53.1)	-0.370 ± 0.411 (-5.4 – 3.6)	18.8 ± 0.43^{c} (15.2 – 23.2)

Table 9.5 - Tree characteristics – pinnule length and asymmetry variables. Mean, standard error and range for all significant variables at the provenance and family level.

Level		Mean ± SE (range)		
Provenance		Absolute FA Not significant	Relative FA Not significant	Pinnule Length Not significant
CH		0.264 ± 0.024 (0.00 – 0.87)	0.077 ± 0.007 (0.00 – 0.27)	3.53 ± 0.092 (2.13 – 5.52)
SE		0.316 ± 0.026 (0.00 – 0.93)	0.086 ± 0.007 (0.00 – 0.33)	3.80 ± 0.083 (1.99 – 5.40)
NW		0.306 ± 0.025 (0.00 – 1.23)	0.084 ± 0.007 (0.00 – 0.26)	3.71 ± 0.093 (2.30 – 6.48)
NE		0.280 ± 027 (0.00 – 1.40)	0.076 ± 0.006 (0.00 – 0.32)	3.67 ± 0.092 (1.77 – 5.18)
Provenance	Family	Not significant	Not significant	Not significant
CH	5	0.272 ± 0.046 (0.00 – 0.87)	0.073 ± 0.013 (0.00 – 0.23)	3.85 ± 0.15 (2.50 – 5.52)
NW	1	0.292 ± 0.039 (0.00 – 0.70)	0.085 ± 0.012 (0.00 – 0.19)	3.52 ± 0.11 (2.60 – 4.67)
NW	3	0.316 ± 0.052 (0.00 – 1.23)	0.077 ± 0.011 (0.00 – 0.24)	4.02 ± 0.196 (2.33 – 6.48)
NE	9	0.294 ± 0.041 (0.00 – 0.70)	0.076 ± 0.010 (0.00 – 0.16)	3.88 ± 0.148 (2.03 – 5.18)
NE	8	0.288 ± 0.053 (0.00 – 1.40)	0.080 ± 0.013 (0.00 – 0.32)	3.59 ± 0.157 (1.93 – 5.13)
SE	10	0.374 ± 0.057 (0.00 – 0.93)	0.101 ± 0.017 (0.00 – 0.33)	3.93 ± 0.177 (1.99 – 5.40)
SE	12	0.264 ± 0.035 (0.07 – 0.67)	0.073 ± 0.010 (0.01– 0.16)	3.72 ± 0.142 (2.1 – 4.9)
SE	11	0.309 ± 0.038 (0.03 – 0.80)	0.085 ± 0.011 (0.01 – 0.22)	3.76 ± 0.114 (2.15 – 4.95)
CH	4	0.250 ± 0.043 (0.00 – 0.77)	0.079 ± 0.014 (0.00 – 0.27)	3.21 ± 0.123 (2.13 – 5.00)
CH	6	0.269 ± 0.036 (0.02 – 0.63)	0.077 ± 0.011 (0.00 – 0.26)	3.52 ± 0.175 (2.30 – 5.21)
NW	2	0.309 ± 0.039 (0.00 – 0.60)	0.089 ± 0.012 (0.00 – 0.26)	3.62 ± 0.155 (2.30 – 5.77)
NE	7	0.251 ± 0.043 (0.00 – 0.75)	0.069 ± 0.011 (0.00 – 0.18)	3.49 ± 0.172 (1.77 – 4.87)

9.3.2 First instar survival and performance

Larval survival averaged 34 % across all trees. Median surivival was 32 %. No significant differences in survival were observed at either the provenance ($F_{3,215} = 2.97$, $p = 0.097$) or family level ($F_{8,215} = 1.28$, $p = 0.262$; Table 9.2). However, survival was significant at the tree level ($F_{94,215} = 1.47$, $p = 0.011$). One tree had 100% survival, and this was from family 2 of the NW provenance. No trees experienced zero survival.

Larval fresh weight averaged 0.395 g and ranged between 0.076 and 1.225 g. Average dry weight was 0.107 g (range: 0.055 – 0.193 g). There was no significant difference between fresh or dry weights of larvae feeding on the different provenances (Fresh: $F_{3\ 214} = 0.41$, $p = 0.753$; Dry: $F_{3,210} = 0.44$, $p = 0.731$; Table 9.6a) or families (Fresh: $F_{8,214} = 1.55$, $p = 0.150$; Dry: $F_{8,210} = 0.89$, $p = 0.530$; Table 9.6b). Furthermore, no significant differences were found for the fresh or dry weights of larvae growing on different trees (Fresh: $F_{94,214} = 1.27$, $p = 0.081$; Dry: $F_{94,210} = 1.04$, $p = 0.408$). The average and median moisture contents of the larvae were similar (average = 67 %, median = 70 %).

Table 9.6a - Insect measurements - Average values and standard errors of survival, fresh and dry weights at the provenance level.

	Larval Measurements Mean ± SE (range)		
Provenance	Survival (%) Not significant	Average Fresh weight/insect (g) Not significant	Average Dry weight/insect (g) Not significant
NW	40.52 ± 3.86 (5.3 – 100.0)	0.371 ± 0.017 (0.099 – 1.225)	0.110 ± 0.003 (0.055 – 0.164)
NE	36.62 ± 2.37 (17.7 – 61.5)	0.362 ± 0.010 (0.107 – 0.638)	0.106 ± 0.003 (0.011 – 0.170)
CH	29.90 ± 2.40 (7.6 – 54.7)	0.363 ± 0.011 (0.087 – 0.601)	0.110 ± 0.003 (0.044 – 0.193)
SE	29.20 ± 3.60 (6.7 – 68.3)	0.344 ± 0.014 (0.076 – 0.680)	0.111 ± 0.003 (0.077 – 0.179)

Table 9.6b - Insect measurements - Average values and standard errors of survival, fresh and dry weights at the family level.

	Larval Measurements Mean ± SE (range)		
Family	Survival (%)	Average Fresh weight/insect (g)	Average Dry weight/insect (g)
	Not significant	Not significant	Not significant
3	45.7 ± 4.47 (33.0 – 75.4)	0.317 ± 0.021 (0.109 – 619)	0.103 ± 0.004 (0.066 – 0.157)
2	45.1 ± 8.9 (11.8 – 100.0)	0.364 ± 0.018 (0.099 – 0.521)	0.106 ± 0.004 (0.055 – 0.144)
7	41.1 ± 4.5 (20.6 – 61.5)	0.366 ± 0.013 (0.205 – 0.478)	0.105 ± 0.006 (0.011 – 0.161)
10	35.5 ± 6.3 (6.8 – 64.6)	0.344 ± 0.024 (0.230 – 0.680)	0.110 ± 0.005 (0.089 – 0.179)
8	34.7 ± 4.0 (17.7 – 55.2)	0.365 ± 0.022 (0.107 – 0.638)	0.109 ± 0.005 (0.070 – 0.170)
9	34.7 ± 3.9 (21.7 – 53.0)	0.357 ± 0.015 (0.0.223 – 0.489)	0.103 ± 0.003 (0.060 – 0.139)
5	33.8 ± 4.1 (11.3 – 54.7)	0.373 ± 0.021 (0.087– 0.571)	0.108 ± 0.004 (0.070 – 0.146)
1	30.3 ± 5.1 (5.3 – 52.7)	0.434 ± 0.037 (0.199 – 1.225)	0.121 ± 0.005 (0.070 – 0.164)
11	29.7 ± 5.7 (8.3 – 52.2)	0.356 ± 0.024 (0.076 – 0.620)	0.115 ± 0.004 (0.086 – 0.162)
4	29.3 ± 4.9 (13.2– 46.0)	0.372 ± 0.017 (0.196 – 0.533)	0.114 ± 0.006 (0.044 – 0.193)
6	26.6 ± 3.5 (7.6 – 40.6)	0.341 ± 0.018 (0.097 – 0.601)	0.104 ± 0.005 (0.062 – 0.176)
12	22.4 ± 6.6 (6.7 – 68.3)	0.330 ± 0.022 (0.119 – 0.526)	0.107 ± 0.004 (0.077 – 0.153)

Multiple stepwise regression showed moisture content and relative FA were both significantly negatively related to survival (Table 9.7).

Table 9.7 - Stepwise multiple linear regression analysis showing significant factors relating to survival of first instar *A. orphana* larvae.

*** Summary of analysis ***

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	0.881	0.44061	7.50	<.001
Residual	105	6.165	0.05872		
Total	107	7.047	0.06586		
Change	-2	-0.881	0.44061	7.50	<.001

*** Estimates of regression coefficients ***

	estimate	s.e.	t(105)	t pr.
Constant	2.193	0.195	11.26	<.001
Moisture	-0.01505	0.00451	-3.34	0.001
Relative FA	-1.732	0.677	-2.56	0.012

9.3.3 Natural defoliation

Natural defoliation was significant at the provenance level ($F_{3,95} = 9.37$, $p = 0.005$; Table 9.2), with the CH provenance experiencing significantly less defoliation (5.6 ± 0.90 %). Significant differences were also observed between families ($F_{8,215} = 9.37$, $p = 0.005$; Table 9.2; Table 9.8). Family 3 from the north-west had the highest defoliation at 75.6%. The three CH families experienced the lowest defoliation.

Table 9.8 - Average defoliation (± SE) by the natural population of *A. orphana*, range and least significant difference for each provenance and family.

Provenance	Family	Mean defoliation ± SE (%) (range) LSD = 24.24
CH		5.6 ± 1.6^{a} (0 – 30)
SE		36.7 ± 5.4^{b} (0 – 95)
NW		50.4 ± 6.4^{b} (0 – 100)
NE		56.7 ± 5.5^{b} (10 – 100)

Family		LSD = 21.30
NW	3	75.6 ± 3.82^{a} (40 – 100)
NE	9	64.4 ± 3.59^{ab} (30 – 100)
NE	8	56.7 ± 5.06^{abc} (20 – 100)
NE	7	48.9 ± 6.82^{bcd} (10 – 90)
SE	10	43.3 ± 3.20^{bcde} (10 – 60)
NW	2	41.3 ± 6.70^{cde} (0 – 90)
SE	11	39.4 ± 6.94^{cde} (0 – 95)
NW	1	34.4 ± 4.97^{de} (0 – 70)
SE	12	27.2 ± 4.95^{ef} (0 – 70)
CH	4	7.8 ± 2.22^{fg} (0 – 30)
CH	6	5.6 ± 1.17^{g} (0 – 20)
CH	5	3.3 ± 0.92^{g} (0 – 10)

Stepwise multiple linear regression showed a* and average pinnule length were significantly and positively related to greater natural defoliation (Table 9.9).

Table 9.9 - Stepwise multiple linear regression analysis showing significant factors relating to natural defoliation of *A. dealbata* trees.

*** Summary of analysis ***

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	10046.	5023.0	5.08	0.008
Residual	105	103825.	988.8		
Total	107	113871.	1064.2		
Change	-2	-10046.	5023.0	5.08	0.008

*** Estimates of regression coefficients ***

	estimate	s.e.	t(105)	t pr.
Constant	-3.4	19.1	-0.18	0.859
a*	4.36	1.88	2.32	0.022
Pinnule length	11.37	5.13	2.22	0.029

Significant correlations between the difference tree and insect characteristics measured were determined from Spearman Rank tests of results averaged across trees (Table 9.10).

Table 9.10 - Significance and relationship of different interacting variables using Spearman Rank statistics.

Correlation	Number	Probability	R^2
ΔHeight vs ΔDBH	108	0.001	0.5795
Moisture vs Nitrogen	108	0.001	0.2929
Moisture vs L*	108	0.01	0.2467
b* vs Dry weight	108	0.01	0.2350
Natural defoliation vs Pinnule length	108	0.02	0.2227
Nitrogen vs Larval fresh weight	108	0.02	0.2220
a* vs Natural defoliation	108	0.03	0.2204
a* vs Survival	108	0.04	0.1945
Moisture vs Natural defoliation	108	0.02	-0.2193
Nitrogen vs Rel FA	108	0.01	-0.2340
b* vs ΔHeight	108	0.01	-0.2418
Moisture vs Survival	108	0.01	-0.2586
Moisture vs a*	108	0.001	-0.3745
a* vs Nitrogen	108	0.001	-0.5583

Ordination showed that all the trees in the trial were very different from each other and there was considerable overlap between provenances. Thus, no clear grouping was apparent except for the survival of larvae feeding on the central highland provenance (Figure 9.5, Figure 9.6). Families were not delineated on these figures, but these also showed overlap and poor grouping. Not all variables were used in the ordination analysis and apart from FA, only those which were found significant in the previous analyses were included. Natural defoliation and survival were found to be on opposite sides of the axes, possibly indicating that different aspects of the trees were having an influence over these (Figure 9.6).

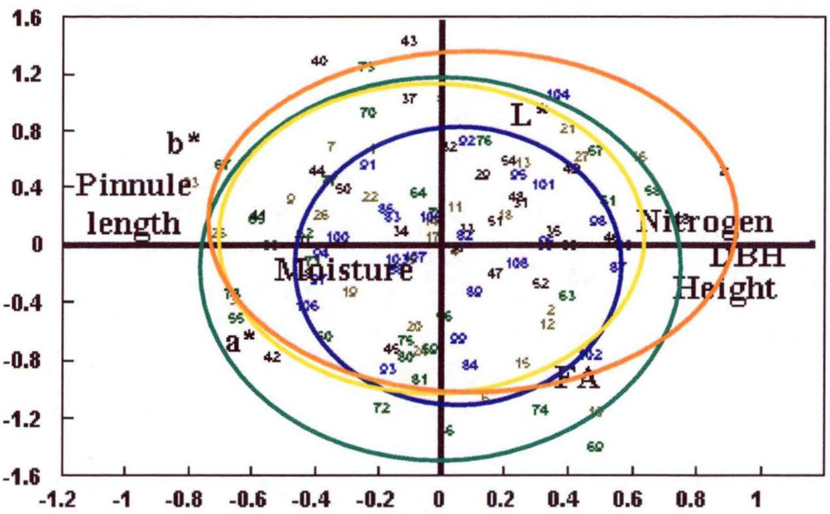


Figure 9.5 - Ordination of 108 trees on the basis of 9 significant variables relating to tree growth, nutrition and morphological characteristics.

Key: Pinnule length = average pinnule length, DBH , Height = differences in diameter at breast height and height over an 11 month period, moisture = foliar moisture content, N = Nitrogen, L* = lightness of the foliage, a* = foliar red/green co-ordinate, b* = foliar yellow/blue coordinate, and FA = relative fluctuating asymmetry. Circles are equivalent to 2 standard deviations around the mean for each provenance: Red = CH, Green = NE, Yellow = NW, Blue = SE.

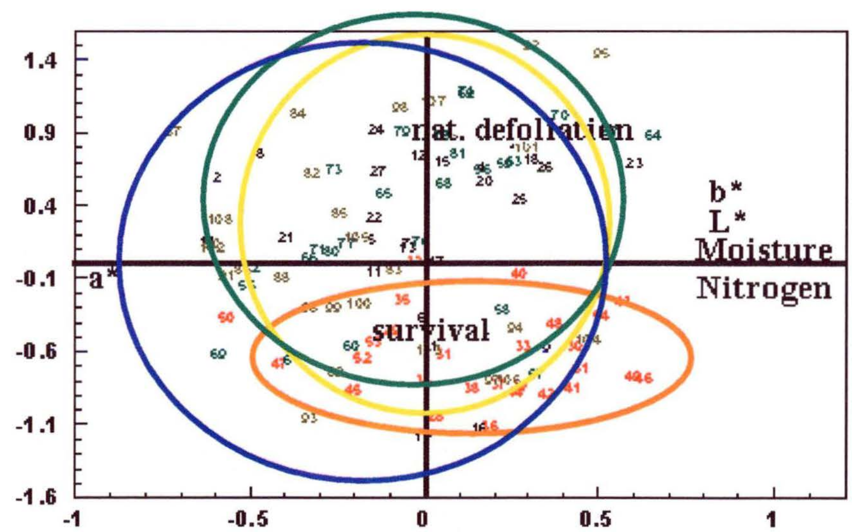


Figure 9.6 - Ordination of 108 trees on the basis of 7 significant variables, relating to tree nutrition, colour, insect survival and natural defoliation.

Key: survival = larval survival, nat. defoliation = defoliation by the natural population, moisture = foliar moisture content, N = Nitrogen, L* = lightness of the foliage, a* = foliar red/green co-ordinate and b* = foliar yellow/blue coordinate. Circles are equivalent to 2 standard deviations around the mean for each provenance: Red = CH, Green = NE, Yellow = NW, Blue = SE.

9.4 Discussion

9.4.1 Tree characteristics

In this chapter, several factors relating to *A. orphana* infestation of *A. dealbata* have been examined. Overall, only four of the 18 variables measured were significant at the provenance level. These four variables were all significantly different in the central highland provenance compared to the other three provenances. The tree characteristics that separated this provenance from the others were lighter coloured foliage, less-red (greener) coloured foliage and higher nitrogen levels. This provenance also experienced significantly less defoliation by the natural *A. orphana* population that was present in the trial area.

On a family level, six of the variables were significant but no distinct trends are apparent (Table 9.3b). Family 10 from the south-east region showed higher volume increments and volume growth rates than all other families. Family 5 from the central highlands had more brown foliage than all other families and also the lowest volume growth rate. Family 5 also experienced the lowest defoliation by the natural population of *A. orphana.*

Many of the variables were found to be significant at the tree level, and the ordination model supported the observations of substantial differences between trees. This finding was not unexpected, due to the outcrossing environment from which the provenance and family seeds were obtained. Greater control over the seed genotype would be necessary to reduce variations between trees.

9.4.2 Larval measurements

Performance of the first instar larvae as measured by survival, fresh and dry weight was not significant at the provenance or family level. This indicates that either there was little difference in the ability of the larvae to obtain adequate nutrition from the foliage of the different provenances and families during the two-month period in the field or, that whilst all trees were acceptable food sources for the larvae, larvae on some trees consumed more poorer quality foliage to obtain the same growth increment as larvae feeding on trees where the foliage was of a better nutritional quality (Miles *et al.*, 1982). The significant difference between trees for larval survival without a corresponding increase in larval weight also supports the idea that larvae may have been consuming more poor quality foliage on some trees compared to others.

The central highland provenance experienced significantly less natural defoliation than the other three provenances. The three families in this provenance were also the three lowest scoring for natural defoliation across all 12 families.

The differences in natural defoliation and larval performance could be attributed to several factors including oviposition preferences, the duration of the experiment and stage used, natural enemies and the distribution of the natural *A. orphana* population.

High natural defoliation was correlated with longer pinnules. Longer pinnules may provide a more protected environment for eggs and developing larvae. As the eggs and larvae in the trial were contained in cloth mesh bags, there was no need for protection from natural enemies and weather. Thus, it could be expected that natural defoliation would not be correlated with survival if this was occurring, a hypothesis that is supported by the results of the trial. This hypothesis is also supported by Patterson *et al.* (1996) who found first instar *C. bimaculata* larvae experienced less mortality due to abiotic factors when enclosed in mesh bags compared to larvae that were exposed.

Natural defoliation was also positively correlated to red foliar colour. Thus, if females are selecting hosts based on visual cues such as colour then they may be selecting for redder foliage. Red foliage was also correlated positively with survival, and thus it is possible that females have evolved to select for hosts which are red coloured, since larvae have higher survival on these hosts. This contrasts with the idea of Courtney and Kibota (1990) that '*mother doesn't know best*' and also the results obtained for *A. orphana* in Chapter 7. Alternatively, Raymond (1998) found that red foliage colour was positively related to defoliation in a study of

C. bimaculata later in the season and suggested that the attraction was possibly due to underlying chemical changes occurring in the trees. In this chapter, high red colour was related to low foliar moisture and nitrogen contents. If ovipositing females are attracted to red coloured foliage, then they are likely to oviposit on trees that are of poorer nutritional quality. The high survival of larvae on these trees may therefore be due to the protection from bags as previously mentioned whilst high natural defoliation may be due to a lesser ability of the trees to recover and send out new shoots (Stone and Bacon, 1994).

Nozzolillo *et al.* (1990) suggested that red foliar colour in *Eucalyptus* trees was due to cold stress which caused an increase in the red pigment, anthocyanin. It is therefore possible that the location of the trial in the Florentine Valley affected the results obtained. The provenances from the north-west, south east and north east were not accustomed to cold winter environments where the minimum temperature is often close to zero, unlike the central highlands provenance. The red foliar colour observed may be a phenotypic symptom of the trial environment and hence the results may not be replicated in other environments. Relative fluctuating asymmetry was also low in the north east and central highlands provenances. These locations both had annual mean temperatures slightly lower than the trial site, whilst the north west and south eastern provenances had mean annual temperatures which were 2.5 °C higher than the trial site.

9.4.3 Plant stress and herbivory

The plant vigour hypothesis predicts insects that feed and oviposit on plants will select for more vigorous or healthier plants (Price *et al.*, 1990; Price, 1991; Price, 1994; Preszler and Price, 1995). In contrast, the plant stress hypothesis suggests that insect herbivores will perform better on stressed plants (White, 1969; Landsberg, 1990a). The relatively poor nutrition of heavily defoliated trees in this study suggests that the plant vigour hypothesis does not apply. However, the plant stress hypothesis does not strictly apply to *A. orphana* either, as this hypothesis considers that trees under stress grow new, high nutrition shoots which benefit insect survival

(Landsberg, 1990b). As discussed in Chapter 8, the timing of the severe defoliation by *A. orphana* does not appear to benefit future stages or generations.

The two preceding hypotheses above measure stress based on chemical characteristics of the trees. In this study, fluctuating asymmetry was used as further measure of environmental stress to complement the growth, nutritional and colour characters examined. Organisms suffering from environmental stress are less symmetrical due to developmental instability (Swaddle *et al.*, 1994; Palmer, 1994). If trees, families or provenances of *A. dealbata* were experiencing environmental stress, then it could be hypothesised that relative fluctuating asymmetry would be significantly less in the central highlands provenance, as this environment is cold and wet, similar to the environment in the Florentine Valley. Thus, the CH provenance trees did not have to adjust to the trial site climate unlike trees from the other provenances, two of which were close to the coast (Figure 9.1). However, whilst asymmetry measurements were taken to assess environmental stress, these were not significantly different and therefore do not support this hypothesis. The CH provenance had the second lowest relative fluctuating asymmetry.

In depth examination of the nutrition, colour and defoliation of the different provenances in their original locations would provide information on whether or not many of the differences observed in this trial are due to climate in the Florentine Valley.

If all significant interactions are summarised, it appears that high first instar survival and recovery occur where foliage is more red in colour, low in nitrogen and low in moisture. The positive relationship between moisture and nitrogen was not observed in Chapter 8, but in this chapter the relationship supports observations of Ohmart *et al.* (1987). Trees low in moisture and nitrogen may also be under nutritional stress, a situation which may ultimately affect levels of important nutrients in the trees. The levels of nitrogen recorded here are still however, higher than the 1.7% determined by Ohmart *et al.* (1987) and thus it is unlikely that this is a major factor affecting the insect survival and defoliation in this study. They are also similar to levels recorded in Chapters 7 (2.57 – 2.92 %N) and 8 (1.37 - 3.32 %N).

Whilst *A. dealbata* was found to have relatively low moisture contents in Chapters 7 (64.4%) and 8 (53 – 73%), the even lower average moisture content of the foliage in this experiment is unexpected. It is possible that even though the moisture content of the foliage is low insects were able to gain adequate moisture from dew and rainfall which collected on the foliage, thus making the actual foliar moisture content relatively unimportant in this trial. If females are selecting trees for oviposition that will promote larval survival and development, then it could be expected that they would chose higher moisture content foliage. Thus, the findings here again support those in Chapter 7 and Berdegue *et al.* (1998) that the insects are ovipositing on a lesser host. Berdegue *et al.* (1998) suggest that a poorer host is used for oviposition to escape enemies, and it is hypothesised here that the cues for oviposition (eg. red colour) are more important than the moisture level of the host when females are selecting oviposition sites. Another possible explanation for the lower moisture content in relation to the other chapters is that the foliage lost moisture after the branch tips were cut from the trees in the field and before they were weighed in the laboratory.

9.4.4 Recommendations for forestry

Acacia dealbata was found to have poor growth form which may have resulted in inaccuracies in many of the growth measurements. In particular, several trees in the trial were double leadering (2 main stems) and often tops were broken from the trees, resulting in a zero or negative height change in the 11 month period of the trial. These problems indicate that an alternative method of measuring growth needs to be determined for this species.

There were no significant differences in the growth rates of the four provenances studied, however the best family for volume increment and growth rate was family 10 from the south-east region. Family 6 from the central highland provenance showed a high volume growth rate as well as relatively low insect survival and damage by the natural *A. orphana* population (5.6%).

The central highland provenance was also the provenance that experienced significantly less defoliation by the natural population of *A. orphana*. Thus, if insect damage was the only consideration, this would be the one recommended for further study. Further examination of the south-east provenance is also recommended due to its relatively high growth rate and moderately low insect damage.

The family that experienced the least damage by the natural population of *A. orphana* was family 5 from the central highland provenance. This family had the fourth highest larval survival rate, although if oviposition were less likely on this family due to colour or nutritive differences then it would still be the optimum family to consider for breeding as it would be less 'selected for' by searching females. Further testing of colour and oviposition behaviour would help to determine if the low damage is due to oviposition inhibition or another factor.

In summary, the central highlands provenance was not the best performing provenance in terms of the growth characteristics, but it was the best performing provenance in terms of damage by the natural *A. orphana* population. Thus, without longer term studies into the trade-off between growth and defoliation it is not possible to determine if this provenance would be the best in terms of plantation growth. Future investigations into double leadering, tip breakage and general growth form in the different provenances may show if this provenance is optimal for plantation conditions.

10. General Discussion

The ability of *A. orphana* to severely damage its hosts has been long recognised (French, 1911; Froggatt, 1923; McKeown, 1942; Elliott, 1978) and it is thought that infestations of *A. mearnsii* by *A. orphana* in the early 1900s may have contributed to the demise of the *A. mearnsii* tanning industry in southeastern Australia (Searle, 1991). Since then, *A. orphana* has received little attention until forestry companies began to consider *A. dealbata* as a plantation species due to its high quality fibre which makes it acceptable for pulp and paper making (Batchelor *et al.*, 1970). It was from this move towards an alternative native species to *Eucalyptus* that this project arose.

10.1 Main findings

The basic biology and ecology of *A. orphana* has now been determined and some factors affecting the interactions of *A. orphana* and its host trees have been identified. This final chapter will integrate the main findings of the preceding chapters, suggest further areas for research and finish with recommendations for forestry management of *A. orphana*.

10.1.1 Lifecycle

This study confirmed the lifecycle described by Elliott (1978) that *A. orphana* has egg, four larval, pre-pupal, pupal and adult stages. Head capsule widths were an effective method of determining instar. *A. orphana* has a lifecycle similar that of the eucalypt-feeding paropsines except that it is strictly univoltine with larvae developing during the cooler winter months. In the summer adults enter an aestivation period.

Developmental studies in the laboratory showed *A. orphana* requires 1266 DD from freshly laid eggs to emergence as adults. The average minimum threshold temperature was found to be 4.4 °C and an upper threshold was found to be approximately 25 °C, as larvae died soon after eclosion at this temperature. It is unlikely that larvae would be exposed to temperatures of 25 °C in winter in Tasmania as the daily average temperature is often below 12 °C, as discussed in Chapter 4.

Field developmental observations showed different results to those obtained at constant temperatures in the laboratory and indicate that for *A. orphana*, laboratory developmental estimates do not apply under field conditions. Applying the laboratory method to the field results suggested that the minimum temperature threshold for the first two larval stages was 1.5 and 1.6 °C. This was lower than predicted in the laboratory and indicates that larvae would develop faster in the field than predicted from the laboratory model. When the field data was modelled using the laboratory minimum threshold of 4.4 °C, the estimate for development was approximately 200DD less than determined in the laboratory. Thus, if forest managers were to use estimates based on laboratory studies, then the timing for chemical implementation would be consistently late.

The reason for the difference in estimates is hypothesised to be primarily due to basking behaviour by the larvae. Maddox (1995) observed that basking resulted in an increase in body temperature of approximately 8°C in *Ps. tigrina.* In this study, it is suggested that *A. orphana* basking behaviour resulted in an increase of body temperature of approximately 3 °C on average. Further laboratory studies utilising fluctuating temperatures and more especially field studies in several different locations would provide evidence of any such difference.

Two peaks of adult activity were observed in the field. The first peak occurred in November, following emergence of adults from pupation and during this peak feeding was observed, but no mating. When the temperature increased, during late December and January, adults 'disappeared' from the field. Whilst it is not known exactly what happened, it is suggested that the beetles entered a quiescence. This quiescence phase lasts through January and ends in the later part of summer, around February. Adult males emerge from the quiescence period approximately one month earlier than females. Following quiescence beetles mate, with oviposition commencing in March.

Mated females in the laboratory were able to lay eggs for an average of 46 days. Unmated females also laid eggs, although these were infertile and clutch sizes were approximately half that of fertile clutches (10 compared to 20 eggs). Lifetime fecundity of female *A. orphana* was low compared to other paropsines (140 compared to 500-1000) (see also Elliott 1978). This relatively low egg number may be partly due to the small adult size of *A. orphana* relative to other paropsines.

10.1.2 Distribution

Fine scale mapping of *A. orphana* distribution was achieved through use of museum records and intensive field surveys. The beetle's distribution was found to be southeastern Australian, extending throughout Tasmania, Victoria and southern New South Wales. *A. orphana* was found in most locations in Tasmania where a host species was present, suggesting that all plantations of *A. dealbata* or *A. mearnsii* developed in Tasmania would be at risk of defoliation.

The distribution of *A. orphana* was modelled in CLIMEX, allowing predictions of the potential distribution (and risk of infestation) throughout the world. Risk predictions are of particular relevance as *Acacia* forestry is an important part of the economy in several third world African and Asian countries.

A downfall of CLIMEX is that modelling an insect with a relatively small distribution such as *A. orphana* in southeastern Australia may result in a greater probability of incorrect predictions. When ecoclimatic index predictions were compared to known defoliation levels at 42 sites, the correlation was not significant, suggesting the model did not accurately predict distribution. Improving accuracy of the model may require input of climatic data from a greater number of locations in southeastern Australia and particularly Tasmania, for which the current model lists only 57 locations.

10.1.3 Host plants

Whilst *A. dealbata* and *A. mearnsii* are widely recognised as hosts for *A. orphana* (Van den berg, 1982; Elliott and de Little, 1985; Bashford, 1991), another potential host identified during this study was *A. baileyana* in Tasmania. The record of *A. orphana* on *A. decurrens* in Victoria (French, 1911) is also confirmed.

The host choice studies conducted for *A. orphana* suggest that *A. mearnsii* was a better food source for larvae than *A. dealbata* in Tasmania. Larvae fed *A. mearnsii* had higher survival and faster development compared to those fed *A. dealbata*. However, oviposition trials did not show *A. mearnsii* was preferred for oviposition in the laboratory. Clearly, field studies are needed to show if *A. dealbata* receives more eggs merely because it is more prevalent in Tasmania as suggested by the '*patch dynamics hypothesis*' (Thompson, 1988). *A. orphana* larvae feeding on *A. dealbata* exhibited an efficiency of conversion of 40%, which is higher that that observed for other *Eucalyptus*-feeding paropsines (Carne, 1966b; S.C. Baker, J.A. Elek and S.G. Candy, unpublished data) and may be due to the higher nitrogen content of the leguminous host (Mattson, 1980; Ernst, 1992).

Bark feeding by larvae of *A. orphana* is an unusual behaviour among paropsine chrysomelids and has been suggested to only occur when foliage is depleted (Elliott, 1978; Elliott and de Little, 1985). However, I recorded this behaviour in early instar larvae fed in petri dishes, and in final instar larvae on potted trees with adequate foliage; suggesting that lack of foliage is not an explanation. Nutrient analyses showed that green stem is lower in nitrogen than foliage and hence could be of lesser nutritional value. Moisture contents were however, similar in the green stem and foliage, and thus there may be little difference in the ability of larvae to feed on these two substrates. Furthermore, as *Acacia* species are leguminous, the nitrogen content of the stem is still relatively high compared to the foliage of *Eucalyptus* spp. Though the stem nitrogen content of *A. dealbata* is lower than the foliage (1.4% compared to approx. 3%), it is only slightly lower that the optimal level of 1.7% N suggested by Ohmart *et al.* (1987) for another paropsine, *P. atomaria.*

Bark feeding may be essential for the sequestering of defence chemicals excreted by *A.* orphana larvae from glands on the 8th abdominal tergite. Larsson *et al.* (1986) found that Scots Pine (*Pinus sylvestris*) trees had more bark consumed by *Neodiprion sertifer* Geoffroy and that the bark was high in resins which were used for defence by the insects. Further investigations into the composition or potency of the defense chemicals when larvae are allowed a diet of foliage only compared to foliage and stem may provide further insight into bark feeding behaviour.

I also tested whether bark feeding was a method by which larvae may have enhanced the trees as a food source for later beetle generations, as suggested by the *'resource regulation hypothesis'* (Craig *et al.*, 1986). However, severe defoliation did not result in higher levels of nitrogen or moisture in the foliage of the recovering trees. Thus, the idea of a partly self-perpetuating problem as discussed by Landsberg (1990b), who suggested dieback in Eucalypt trees was due to the continual feeding of insects on re-growth foliage does not appear to be the explanation for the bark feeding or severe defoliation caused by *A. orphana.*

Examination of defoliation and survival of first instar larvae in the provenance trial showed that some intraspecific variation (or resistance) may be present within *A. dealbata.* The provenance from the central highlands experienced significantly less natural defoliation. This provenance also had significantly higher foliar nitrogen levels and significantly lighter and less red coloured foliage than the other three provenances. In another paropsine system, red foliar colour was associated with increased oviposition (Raymond, 1998). Whilst it was not tested here, foliage colour may be a visual cue for *A. orphana* when selecting hosts for oviposition and may be partly responsible for the low levels of natural defoliation scored for the central highlands provenance.

10.2 Recommendations for Forestry

This thesis provides a foundation for a management program for *A. orphana* in Tasmania. The first key element of a management program is monitoring. I recommend the use of a beating tray and stick (as utilised in chapter 4). This equipment provides a fast and effective method of collecting adults and larvae of all stages. This method is therefore more appropriate for *A. orphana* sampling by industry workers than searching the foliage for the different stages.

Monitoring should commence in March, as this is the start of the larval development period. It would also enable any discrepancies in the timing of the beetles' development (due to basking) to be detected and accounted for. The field model predicts third instars will be present at approximately 216 DD > 4.4 °C from the peak of the first instar. The higher threshold of 4.4 °C is recommended rather than the lower field-derived threshold (see Chapter 4) as the lower threshold is based on relatively few values and requires further field investigation for confirmation. Sampling at weekly intervals during March with a beating tray would enable determination of the peak of the first instar stage, and hence it would be possible to predict the time when third instar larvae would be present in the field. The peak of the first instar is suggested as a starting point for the accumulation of DD, as this point is easier to determine in the field than the egg stage. The third instar is recommended as the stage for management measures as this stage is present when damage becomes apparent in the field. Also, it is likely that considerable natural mortality would have occurred by this stage of larval development. Furthermore, this stage is present towards the end of winter, and rainfall may be less likely to reduce the efficacy of sprays.

A priority for future research should be to determine an economic threshold for *A. orphana* damage to *A. dealbata*. In particular, evaluation of losses in volume increment of damaged trees compared to undamaged trees is important to determine if management of *A. orphana* is economically viable.

Acacicola orphana is widespread throughout Tasmania. This indicates that *A. dealbata* and *A. mearnsii* plantations in any area of the state are likely to become infested and suffer either partial or severe defoliation. The predictive model suggests that this statement holds also for the southeastern region of mainland Australia. It is therefore suggested that *A. dealbata* plantations be considered in lower risk areas such as Western Australia, where *A. orphana* has not become established.

The presence of natural enemies of *A. orphana* in the field environment did not have a significant impact on population levels. Exploration into *Beauveria bassiana* as a spray application for adults during November or December when adults are present on the foliage may be a worthy investigation. The weather at this time of year is more conducive to products such as this, as rainfall is declining with the onset of summer and there is less chance of the product being washed from the foliage. If adults were infected with the fungus, they would carry it during their aestivation period and fail to emerge in the late summer. This would effectively reduce the number of adults laying eggs and hence the size of the next generation.

As *A. orphana* is widespread in Tasmania, the planting of less susceptible host trees should be considered. Of the two main host *Acacia* species examined, both were similarly defoliated in field conditions. If damage is the only consideration, then the species with the best pulping characteristics (eg. *A. dealbata* rather than *A. mearnsii*) would be optimal. Within this species, the provenance study showed significant differences between the four provenances examined. In particular, the provenance receiving the lowest natural defoliation (of only 5-10%) was the central highland provenance. Further evaluation of the other 24 provenances in the greater provenance trial may provide provenances with even less susceptibility.

In the study here, natural defoliation was poorer on trees with shorter pinnules. Hence, breeding and selection of trees with shorter pinnules may be one method of reducing damage. The observation that trees with more red (higher a*) coloured foliage suffered greater defoliation is also important. Thus, foliar colour assessment could be another method by which industry could determine if trees in particular areas are more or less susceptible to *A. orphana*.

10.3 Genes versus the environment and insect resistance breeding

The central highland provenance of *A. dealbata* experienced less damage by *A. orphana*. The reduced damage in the field compared to the other provenances was hypothesised not to be solely due to genetic influences, although a genetic basis existed. I suggest that the apparent resistance was due partly to the climate of the trial area. Further evaluation of the genotypes at multiple sites would confirm this hypothesis. Traditionally, plant breeding has focussed on the improvement of a few traits in selected plants, commonly growth rates or productivity and occasionally insect damage reduction (Smith, 1992; Borralho *et al.*, 1993; Farrow *et al.*, 1994; Leather, 1996; Soria and Borralho, 1997; Ehlers and Hall, 1997; Ranalli and Cubero, 1997; Sachs *et al.*, 1998; Stone, 2000). However, the importance of abiotic factors on the expression of genetic resistance is difficult to assess, resulting in relatively few authors reporting in any detail when the confounding effects of climate have impacted on their heritability studies. It has been demonstrated however, that tree species are more resistant to insect attack if they are grown in an environment for which they have been selected. For example, Soria and Borralho (1997) found that two *E. globulus* clones, phenotypically selected for growth and health in the Spanish climate, had better growth and resistance to *Phoracantha semipunctata* Fabricius than other seedlings not as well adapted to that environment. Also, Hanks *et al.* (1995b) observed that of several *Eucalyptus* species examined in California, species more resistant to *P. semipunctata* were those that originated from a climate similar to that in which the trial was conducted. One exception to this hypothesis is that of Farrow *et al.* (1994) who observed that whilst *E. globulus* subsp. *bicostata* (Eurrabie) was selected for its drought and cold tolerant properties in SE Australia, it was also very susceptible to *Mnesampela privata* Guenée.

Tingey and Singh (1980) discuss in detail a range of environmental factors that influence the magnitude and expression of host-plant resistance. They cite several examples (eg. Dahms and

Painter, 1940; McMurtry, 1962; Schalk *et al.*, 1969; Foster, 1976) where temperature changes affect resistance and suggest that the interpretations of host-plant interactions, in particular resistance effects, need to be made with consideration to temperature. Light and relative humidity can also alter fundamental plant processes as well as affecting insect development (as discussed in Chapter 4). A genotype-light interaction has been presented as an explanation for reduced resistance of potatoes (due to decreased concentrations of total glycoalkaloids in shaded potatoes) to the Colorado potato beetle, *Leptinotarsa decemlineata* (Pierzchalski and Werner, 1958 – cited by Tingey and Singh (1980)). Light availability also affected the defense chemicals of tomatoes, such that caterpillars were heavier and consumed more foliage of shaded plants which were lower in allelochemicals (Jansen and Stamp, 1997).

The effect of host health, which is related to soil fertility and nutrition as well as climate, can also lead to changes in the expression of genetic resistance. Sachs *et al.* (1998) noted that moisture and fertility impacted on the expression of the *cryIA(b)* (insect-resistance) gene in cotton and this highlights the need for further research into specific environmental factors and their effect on gene expression.

Raymond (1995) found a foliar colour cffcct rclating to damagc by *C. bimaculata*, but no effect due to family. Nozzolillo *et al.* (1990) record that foliar colour changes may be due to abiotic effects that cause changes in leaf chemistry. This observation is supported in part by work presented in this thesis.

Abiotic effects are unpredictable and may favour the plant or insect unequally, thereby affecting the expression of genetic resistance by an unknown amount. After finding that moisture and fertility reduced the expression of an insect-resistance in cotton, Sachs *et al.* (1998) suggested further experimentation be conducted in uniform conditions to reduce the potentially confounding environmental effects. But, if the expression of the gene is variable due to climatic conditions, then the value of this suggestion is questionable. Without extensive testing in different environmental conditions, genetic technology efforts to improve crop productivity and insect resistance may ultimately be confounded by abiotic factors and insects, which are often not affected by genetic boundaries (except when antibiosis is occurring).

In the cotton industry in the United States, early maturity has been utilised for reduction of cotton boll weevil losses since the 1800s (Smith, 1992). Insect damage has also been reduced via enhanced plant nutrition, coordinated planting and chemical application times on a district scale (Smith, 1992). Furthermore, chemical termination of crop growth is followed by fast destruction of stalks to reduce the insect population (Kittock *et al.*, 1973; Walker and Smith, 1993). A similar 'big picture' approach involving cultural, chemical and genetic resistance control mechanisms has been taken to manage the squash bug, *Anasa tristis,* which damages cucurbits (Margolies *et al.*, 1998). Leather (1996) commented that '*tree breeding is generally conducted by silviculturalists who rarely consider the insect component of the environment in which trees are growing; correspondingly, many entomologists fail to consider the plant component of the interaction*'. In forestry, a 'big picture' approach is important, and even more necessary is a well-coordinated effort for the management of pest problems through the consideration of factors affecting both insects and plants. Stone and Bacon (1995) suggested that trees with vigorous growth were less affected by herbivory, due to a smaller portion of canopy being lost. Promotion of site-specific tree selection as well as greater tree health and vigour in resource-poor environments would reduce the impact of herbivore damage (Stone and Clarke, 1998; Stone, 2000). Enhancement of natural insect resistance in trees through the use of both genetics and environmental matching would benefit industry in several ways. For example, natural insect resistance would reduce the need for chemicals, be active at all levels of insect infestation, reduce environmental contamination and fit in well with other parts of the integrated pest management program (Leather, 1996). To manage *A. orphana* in *A. dealbata* plantations, it is suggested that genetic, environmental and cultural factors be examined. In particular, further research in the following areas is suggested;

1. Evaluation of an economic threshold, trading infestation levels against productivity losses.

2. Continuation of provenance/family studies as commenced in chapter 9 and further examination of provenance resistance to insect damage in relation to climate adaptation.

3. Examination of *Beauveria bassiana* as a potential control and cost/benefit analyses.

4. Investigation into oviposition cues. Larvae do not move between trees unless foliage is touching. If oviposition cues can be reduced, there may be possible to reduce damage. However, this suggestion carries the risk of forcing host changes by the insect over time.

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Appendix 1

Biology of the Paropsini (Chrysomelidae: Chrysomelinae)

Tara L. Simmul[1] and David. W. de Little[2]

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Simmul, T. L., de Little, D, W., Biology of the Paropsini (Chrysomelidae: Chrysomelinae), in Advances in Chysomelidae Biology 1, Backhuys Publishers, M. L. Cox (ed), Leiden, The Netherlands, pp. 463-477. ISBN 9057820285 (1999)

Appendix 2

PARASITISM OF *ACACICOLA ORPHANA* (ERICHSON) (COLEOPTERA: CHRYSOMELIDAE) IN TASMANIA

Tara L. Simmul[1] and Anthony R. Clarke[2]

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Simmul, T. L., Clarke, A. R., 1999, Parasitism of Acacicola orphana (Erichson) (Coleoptera: Chrysomelidae) in Tasmania, Australian Entomologist, 26(3), 87-90

Appendix 3 – Original description of *A. orphana* (Erichson, 1842).

Pallide testacea, elytris striato-punctatis, laete flavis, interstitiis subtilissime punctulatis, primo ante medium, 4. *Et* 6. Basi apiceque fusco-lineolatis – Long.2 ½ lin.

Oblonga, convexa, pallide testacea, nitida. Antennae nigrae, articulis 4 primis flavis. Caput vage subtiliter punctatum. Thorax brevis, lateribus subrotundatis, integris, parce subtilissimeque punctatus, lateribus punctis maioribus immixtis. Scutellum laeve. Coleoptera modice convexa, striato-punctata, interstitiis parce omnium subtilissime punctulatis, margine laterali crebre fortiterque punctato, laete flava, interstitio 1.ante medium, 2. Antice posticeque, 6. Antice, 7. Postice litura fusca, prima prope suturam longiore, anterioribus brevissimis.

Appendix 4 – Botanical descriptions of *A. dealbata* and *A. mearnsii.*

(From Searle 1997)

Acacia dealbata Link

Small erect tree from 2 m in sub-alpine provenances (where often distorted) up to straight forest trees 35 m high in sheltered lowland habitats; trunk 5-75 cm diam.; crown usually open and up to 10m across, bark grey or silver-grey, often mottled, smooth; branchlets angular, shortly pubescent, foliage usually glaucous, silvery, rarely otherwise; leaves bipinnate, 2.5-15 cm long and 4-10 cm wide; petiole 0.5-3 cm long; rachis 2-12 cm long with a small but prominent hairy gland on the upper surface near the base of each pair of pinnae with no additional glands between them; pinnae 8-25 pairs; pinnules 17-50 pairs, close-set and sometimes overlapping one another, mostly 2-5mm long, rarely less, linear oblong, obtuse or acute, at least minutely pubescent with more or less appressed hairs; inflorescence in axillary racemes (and sometimes in panicles) of 3-10 flower heads; flower-heads 5-10 mm diam., globular, pale- to bright-yellow, not always conspicuous, fragrant, 25-35-flowered; peduncles pubescent, about 6 mm long; flowers 5-partite; pods 48 cm long and 8-12 cm wide, flat, straight or sometimes twisted, slightly constricted between the seeds, glabrous, usually purple-brown and sometimes glaucous; seeds longitudinal in the pod; funicle short, expanded into a small terminal aril. Flowering from late winter to late spring.

Indigenous to New South Wales, Australian Capital Territory, Victoria and Tasmania.

Acacia mearnsii De Wild.

Small spreading tree 5-15 m high and nearly the same across; trunk up to about 35 cm diam.; bark dark-grey, smooth but sometimes tending to be rough on the lower parts of older trunks; branchlets angular, pubsecent; foliage dense, dark green when mature but often honey- or golden-brown in colour when young; leaves bipinnate, 5-15 cm long and 4-12 cm wide; petiole 1-5 cm long; rachis 5-15 cm long with a prominent hairy gland on the upper surface near the base of each pair of pinnae and with one or more additional hairy glands between at least two pairs of pinnae along the rachis; pinnae 10-25 pairs; pinnules about 30-65 (occasionally as few as 15 on pinnae near the base of the rachis) pairs, close-set and often overlapping one another, 1.2 – 4 mm long and 0.5-1.0 mm wide, linear oblong, obtuse rarely otherwise, glabrous above and pubescent below; inflorescence in axillary racemes (and sometimes in panicles) of 3-10 flower heads; flower-heads 5-10 mm diam., globular, pale-yellow, 20-30-flowered, fragrant; peduncles usually golden pubescent; flowers 5-partite; pods 4-15 cm long and 5-8 mm wide, flat, straight, somewhat constricted between the seeds, glabrous, purple-brown; seeds longitudinal in the pod; funicle short, expanded into a more or less oblique aril. Flowering from late spring to early summer.

Indigenous to south-eastern Australia; New South Wales, Australian Capital Territory, Tasmania and Victoria.

Appendix 5 – Recipe for Glycerine Jelly

Glycerine jelly consists of water (6 parts), glycerine (7 parts) and gelatine (1 part). These are mixed together and then heated in a water bath for 15 minutes. The jelly sets as it cools and maintained the leaflets and pinnules in good condition until measurement.

Appendix 6 – Example of multiple stepwise linear regression

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***** Regression Analysis *****

 Response variate: relFAcor
     Fitted terms: Constant, a, b, L, Moist, N, voldiff


 *** Summary of analysis ***

              d.f.        s.s.        m.s.      v.r.  F pr.
 Regression     6      0.00763   0.0012714      1.42  0.217
 Residual      95      0.08535   0.0008984
 Total        101      0.09298   0.0009206

 Change        -6     -0.00763   0.0012714      1.42  0.217

 Percentage variance accounted for 2.4
 Standard error of observations is estimated to be 0.0300
 * MESSAGE: The following units have large standardized residuals:
                  84        2.65
 * MESSAGE: The following units have high leverage:
                  32       0.231
                 108       0.239


 *** Estimates of regression coefficients ***

                  estimate          s.e.     t(95)  t pr.
 Constant           -0.011         0.107     -0.10  0.920
 a                 0.00341       0.00402      0.85  0.399
 b                 0.00010       0.00179      0.06  0.955
 L                 0.00106       0.00189      0.56  0.576
 Moist           -0.000878      0.000639     -1.37  0.173
 N                  0.0209        0.0142      1.48  0.143
 voldiff         0.0000936     0.0000468      2.00  0.048


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 ***** Regression Analysis *****

 Response variate: relFAcor
     Fitted terms: Constant, a, L, Moist, N, voldiff


 *** Summary of analysis ***

              d.f.        s.s.        m.s.      v.r.  F pr.
 Regression     5      0.00763   0.0015251      1.72  0.138
 Residual      96      0.08536   0.0008891
 Total        101      0.09298   0.0009206

 Change        -5     -0.00763   0.0015251      1.72  0.138
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Percentage variance accounted for 3.4
Standard error of observations is estimated to be 0.0298
* MESSAGE: The following units have large standardized residuals:
                   84        2.66
* MESSAGE: The following units have high leverage:
                  108        0.238


*** Estimates of regression coefficients ***

                   estimate          s.e.     t(96)  t pr.
Constant             -0.010         0.104     -0.09  0.926
a                   0.00340       0.00400      0.85  0.397
L                   0.00108       0.00183      0.59  0.556
Moist             -0.000876      0.000635     -1.38  0.171
N                    0.0208        0.0140      1.49  0.140
voldiff           0.0000935     0.0000465      2.01  0.047


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***** Regression Analysis *****

 Response variate: relFAcor
     Fitted terms: Constant, a, Moist, N, voldiff


*** Summary of analysis ***

              d.f.         s.s.         m.s.       v.r.  F pr.
Regression       4      0.00731    0.0018286       2.07  0.091
Residual        97      0.08567    0.0008832
Total          101      0.09298    0.0009206

Change          -4     -0.00731    0.0018286       2.07  0.091

Percentage variance accounted for 4.1
Standard error of observations is estimated to be 0.0297
* MESSAGE: The following units have large standardized residuals:
                   84        2.60
* MESSAGE: The following units have high leverage:
                   32        0.127
                  108        0.225


*** Estimates of regression coefficients ***

                   estimate          s.e.     t(97)  t pr.
Constant             0.0477        0.0380      1.26  0.212
a                   0.00149       0.00235      0.63  0.528
Moist             -0.000892      0.000632     -1.41  0.161
N                    0.0169        0.0123      1.38  0.172
voldiff           0.0000881     0.0000454      1.94  0.056


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***** Regression Analysis *****

 Response variate: relFAcor
     Fitted terms: Constant, Moist, N, voldiff
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*** Summary of analysis ***

                d.f.        s.s.         m.s.       v.r.  F pr.
Regression        3      0.00696    0.0023199       2.64  0.054
Residual         98      0.08602    0.0008778
Total           101      0.09298    0.0009206

Change           -3     -0.00696    0.0023199       2.64  0.054

Percentage variance accounted for 4.7
Standard error of observations is estimated to be 0.0296
* MESSAGE: The following units have large standardized residuals:
                  84        2.60
* MESSAGE: The following units have high leverage:
                  32        0.121
                 108        0.217


*** Estimates of regression coefficients ***

                  estimate          s.e.     t(98)  t pr.
Constant            0.0624        0.0299      2.08  0.040
Moist            -0.001028      0.000593     -1.73  0.086
N                   0.0129        0.0105      1.23  0.222
voldiff          0.0000860     0.0000452      1.90  0.060
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***** Regression Analysis *****

 Response variate: relFAcor
     Fitted terms: Constant, Moist, voldiff


*** Summary of analysis ***

                d.f.        s.s.         m.s.       v.r.  F pr.
Regression        2      0.00563    0.0028168       3.19  0.045
Residual         99      0.08735    0.0008823
Total           101      0.09298    0.0009206

Change           -2     -0.00563    0.0028168       3.19  0.045

Percentage variance accounted for 4.2
Standard error of observations is estimated to be 0.0297
* MESSAGE: The following units have high leverage:
                 108        0.217


*** Estimates of regression coefficients ***

                  estimate          s.e.     t(99)  t pr.
Constant            0.0860        0.0231      3.72  <.001
Moist            -0.000836      0.000574     -1.46  0.148
voldiff          0.0000882     0.0000453      1.95  0.054
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